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# RECENT ADVANCES IN BACTERIOLOGY

AND THE STUDY OF THE INFECTIONS

BY

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With 22 Illustrations



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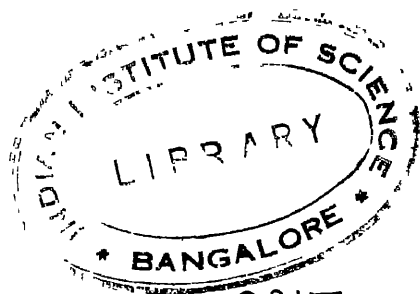
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## PREFACE

THE attempt to write a book upon "Recent Advances in Bacteriology" may be looked upon as a bold undertaking, especially when it is made by a single individual. In none of the subjects ancillary to medicine is knowledge in a detailed form being so widely and rapidly advanced, whilst in its wider application the science of bacteriology is extending to-day into many fields far removed from medicine. Perhaps, however, this very complexity warrants the effort which is made here—an attempt by a general reader and student of medical bacteriology to present in a readable form some of the more recent changes in the subject, and to indicate the lines upon which it is evolving.

One of the difficulties which has faced the author has been for what type of reader this book should be written. It is obviously not within his competence to write with authority upon more than a small portion of the wide field here embraced, and in every subject treated those who are engaged at the moment in its investigation will probably find good cause to cavil at its presentation. The endeavour has been made to take a broad view of many subjects, and, in keeping the balance between extreme technicality and what is already common knowledge, to present a readable exposition of recent work which the general medical reader, not himself especially versed in bacteriology, may appreciate. At the same time the author seeks to indicate to those who do possess some knowledge of bacteriology and the infections what is being done in spheres outside of their own.

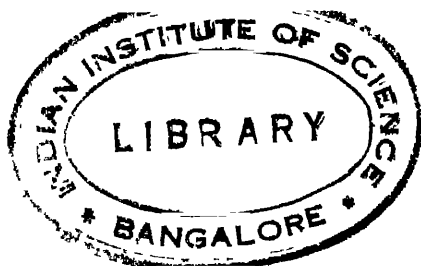
The writer has, perforce, set out a good deal of matter upon which at the present time judgment is suspended, and views which are undoubtedly speculative. He disclaims all responsibility for the accuracy of quoted work. At times, and where it has seemed desirable, he has endeavoured to place before his readers a judicial summing up of moot questions.

A further difficulty has lain in the rapid evolution of the subject. This has made it impossible to take notice of all of the most recent work, since the business of publication necessitates that a limit must be set somewhere. The period covered is, therefore, roughly that from the commencement of the War to the beginning of 1928, with certain excursions into the past for historical purposes.

The author desires to thank his former assistant, Dr Joan Ross, for the drawings from which a number of the figures have been produced. The source of other illustrations is acknowledged, and thanks are due to the respective authors and publishers for permission to reproduce them.

J. H. D

CARDIFF.



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# BACTERIOLOGY

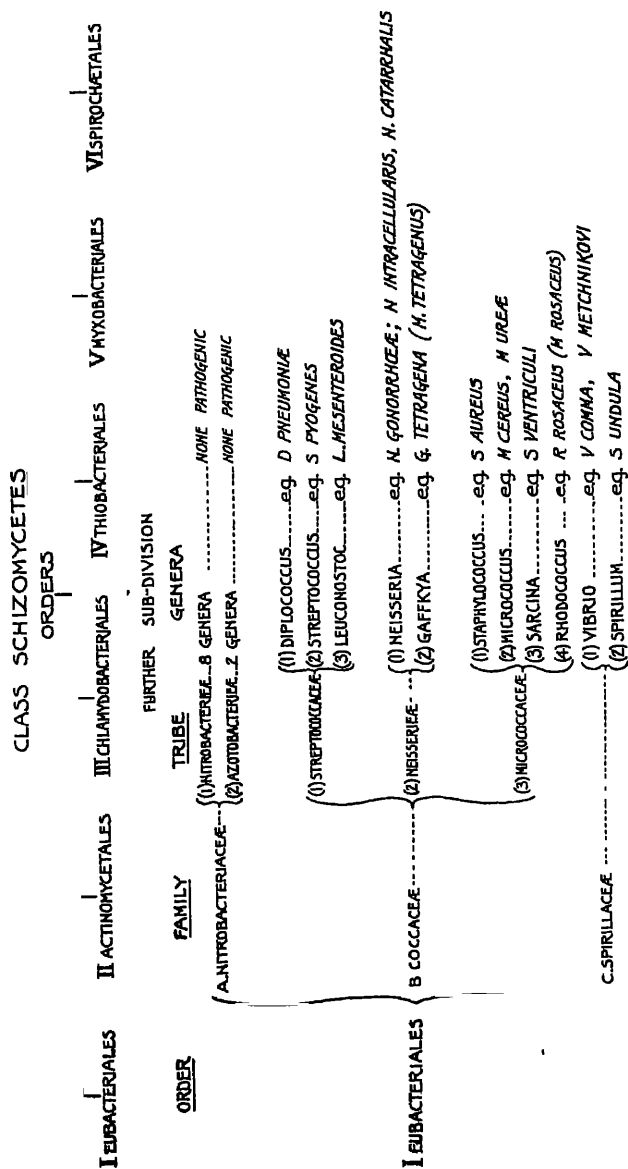
## CHAPTER I

### THE CLASSIFICATION OF BACTERIA

BACTERIAL nomenclature has long been in an admittedly unsatisfactory state. Most medical bacteriologists employ only a few names in their terminology and, knowing what they mean by them, are but little interested in the efforts of the systematists who, approaching the subject from a rather different angle, have evolved systems which the medical worker finds unnecessary and rather cumbersome. The latter frequently sees no irritating anomaly in the classification of the tetanus, anthrax, and influenza organisms in one and the same genus ; whilst the former, for his part, is not concerned with, and often being a botanist does not appreciate, those serological differences within a single species which in medicine may be so important.

The older classifications were almost wholly morphological, down to the well-known systems of Lehmann and Neumann (1896), Migula (1900) and Chester (1901) More recently the impossibility of adherence to this usage has been recognised, and the systems of Winslow (1908), for the coccaceæ, and of Orla-Jensen (1908), Buchanan (1917), Castellani and Chalmers (1919), and others, have been in large part based upon biological properties.

In 1917 a Committee on Characterisation and Classification, of the Society of American Bacteriologists, evolved a scheme of classification largely based upon those of Winslow, Castellani and Chalmers, and Buchanan. This system has been extended by Bergey and his collaborators, in their well-known book, to embrace the characters of the individual species of the genera recognised



# THE CLASSIFICATION OF BACTERIA

3

(ORDER)	(FAMILY)	(TRIBE)	(GENERA)
EUBACTERIALES (CONTINUED)	D. BACTERIACEAE	(1) OHRMOMYXACEAE	(1) SERRATIA.....eg. <i>S. MARCESCENS</i> ( <i>M. PRODIGIOSUS</i> )
		(2) FLAVOBACTERIUM	(2) FLAVOBACTERIUM.....eg. <i>C. VIOLACEUM</i> ( <i>B. VIOLACEUS</i> )
		(3) CHROMOBACTERIUM	(3) CHROMOBACTERIUM.....eg. <i>D. AERUGINOSA</i> ( <i>B. PROTEANUS</i> ), <i>P. FLUMESCENS</i> ( <i>B. FLUMESCENS LIQUIFACTUS</i> )
		(4) PSEUDOMONAS	(4) PSEUDOMONAS.....eg. <i>C. CELLULOSE</i> DIGESTING BACTERIA
		(5) MICROBACTERIACEAE	(5) MICROBACTERIACEAE.....eg. <i>A. LIQUEFACENS</i> , <i>A. METALLICIGENES</i> ( <i>B. LACTIS VISCOSUM</i> )
		(6) LACTOBACILLACEAE	(6) LACTOBACILLACEAE.....eg. <i>L. BOAS-OPPLER</i> , <i>L. BULGARICUS</i> , <i>L. ACIDOPHILUS</i>
		(7) ERWINIACEAE	(7) ERWINIACEAE.....eg. <i>E. AMYLOPHILA</i> ( <i>BACILLUS OF APPLE BLIGHT</i> )
		(8) KURTTHIACEAE	(8) KURTTHIACEAE.....eg. <i>P. HYACINTHI</i>
		(9) ISCHERICHIA	(9) ISCHERICHIA.....eg. <i>K. ZENKERI</i> ( <i>PROTEUS ZENKERI</i> )
		(10) AEROBACTER	(10) AEROBACTER.....eg. <i>E. COLI</i> ( <i>B. COLI</i> ), <i>E. COMMUNIOR</i> , <i>E. ACIDI LACTIS</i>
		(11) PROTEUS	(11) PROTEUS.....eg. <i>A. AEROGENES</i> ( <i>B. LACTIS AEROGENES</i> )
		(12) SALMONELLA	(12) SALMONELLA.....eg. <i>S. ABORTUS-EQUI</i> , <i>S. SCHOTTMÜLLERI</i> ( <i>PARA B.</i> ), <i>S. PARATYPHI</i> ( <i>PARA A.</i> )
		(13) EBERTHELLA	(13) EBERTHELLA.....eg. <i>E. TYPHI</i> ( <i>B. TYPHOSUS</i> ), <i>E. DISSEMINATA</i> ( <i>SING.</i> ), <i>E. PARATYPHI</i> ( <i>FLAHER-Y</i> )
		(14) ALKALIGENES	(14) ALKALIGENES.....eg. <i>A. FACILIS</i> , <i>A. BRUNNISEPTICUS</i> , <i>A. ABORTUS</i> , <i>A. HELIOTENSIS</i>
		(15) PASTEURIELLE	(15) PASTEURIELLE.....eg. <i>P. ANTIMONIA</i> ( <i>PARA CHOLERA</i> ), <i>P. COMOLICHA</i> ( <i>B. LEPIDOTICUM</i> ), <i>P. PESTIS</i> , <i>P. TULARENSIS</i>
BACILLACEAE	BACILLACEAE	(16) KLEBSIELLE	(16) KLEBSIELLE.....eg. <i>K. PNEUMONICAE</i> ( <i>PNEUMONICUS</i> ), <i>K. RHINOSCLEROMYXITIS</i>
		(17) NECHROMPHILUS	(17) NECHROMPHILUS.....eg. <i>H. INFLUENZAE</i> , <i>H. PERTUSSIS</i> , <i>H. DUCKREYI</i>
		(18) DIALISTER	(18) DIALISTER.....eg. <i>D. PNEUMONICUS</i> ( <i>OLITSKY AND GATES</i> ) [ <i>ANEROBIC</i> ]
		(19) BACTEROIDES	(19) BACTEROIDES.....eg. <i>B. FRAGILIS</i> , <i>B. BIFIDUS</i> [ <i>ANEROBIC</i> ]
		(20) BACILLUS	(20) BACILLUS.....eg. <i>B. SUBTILIS</i> , <i>B. ANTHRACIS</i> , <i>B. THERMOPHILUS</i>
		(21) CLOSTRIDIUM	(21) CLOSTRIDIUM.....eg. <i>C. WELCHII</i> , <i>C. BOTULINUM</i> , <i>C. TETANI</i> , <i>C. EDENTIS-MALIGNI</i> ( <i>V. SEPTIQUE</i> )

## THE CLASSIFICATION OF BACTERIA

(ORDER)	(FAMILY)	(TRIBE)	(GENERA)
II ACTINOMYCETALES	A. ACTINOMYCETACEAE		(1) ACTINOBACILLUS... eg. A. LIGNIERESI
			(2) LEPTOTRICHIA... eg. L. BUCCALIS
			(3) ACTINOMYCES... eg. A. BOVIS, A. HOMINIS, A. MADURÆ, A. ASTERINOIDES
			(4) ERYTHROPELOTHRIX... eg. E. RHUSIDIOPTILAE (BACILLUS OF SWINE ERYTHROPELAS)
	B. MYCOBACTERIACEAE		(1) MYCOBACTERIUM... eg. M. TUBERCULOSIS (B. TUBERCULOSIS), M. SHEGGHATIS
			(2) CORNYNEBACTERIUM... eg. C. DIPHTHERIE; C. PSEUDODIPHTHERIE; C. OVIS (B. PSEUDOTUBERCULOSIS OVIS)
			(3) FUSIFORMIS... eg. F. DENTITUM (WITH BORRELLIA IN VINCENT'S ANGINA)
			(4) PFEIFFERELLA... eg. P. MALLEI (B. MALLEI)
III CHLAMYDOBACTERIALES	CHLAMYDOBACTERIACEAE		IRON AND SWAMP-WATER BACTERIA NONE PATHOGENIC
IV THIOBACTERIALES	A. RHODOBACTERIACEAE B. BEEGLIATOACEAE C. ACHROMATIACEAE		BACTERIA CONTAINING SULPHUR GRANULES AND/OR BACTERIORED PURPUREUM NONE PATHOGENIC
V MYCOBACTERIALES	MYCOBACTERIACEAE		FORMING PLASMIDIUM-LIKE MASSES IN DUNG AND UPON DECAYING VEGETABLE MATTER NONE PATHOGENIC
VI SPIROCHETALES	SPIROCHETACEAE		(1) SPIROCHETA... eg. S. PALLIDUM
			(2) SAPROSPIRA... eg. S. GRANDIS
			(3) CRISTISPIRA... eg. C. BALBANI
			(4) BORRELLIA... eg. B. RECURRENTIS (SPIRILLUM RECURRENTIS) B. VINCENTI
			(5) TREPONEMA... eg. T. PALLIDUM
			(6) LEPTOSPIRA... eg. L. ICTEROHÆMORRHAGICÆ

by this committee. In this classification the International Rules of Botanical Nomenclature have been adopted in so far as they can be made to apply to bacteriology.

Bergey's manual, which was published in 1924, and issued in a second edition in 1926, represents a serious effort to unify bacterial classification and to combine the points of view of the general and medical bacteriologists. Although many of the names employed are excessively cumbersome—a result of constant concessions to priority and botanical usage—and whilst the system can present no finality in so rapidly changing a subject, it is undoubtedly a step in the right direction. Whilst the medical bacteriologist will no doubt continue to cling to certain of his time-honoured terms, such as *Bacillus typhosus* and *pneumococcus*, there is plenty of evidence in recent bacteriological literature that the nomenclature adopted by the Society of American Bacteriologists is creeping into current usage. The relationships of the various Families, Tribes and Genera recognised in this classification are not easy to follow when scattered through the pages of a manual, and we have therefore compiled the tabular statement on pp. 2—4, in order to make these points clear and to indicate, in a broad way, what are the new guises under which old friends may be cloaked.

### REFERENCES

- "Bergey's Manual of Determinative Bacteriology" Second Edition.  
The William and Wilkins Co, Baltimore, 1926  
R E BUCHANAN "General Systematic Bacteriology" *Ibid*, 1925.  
(This volume contains a full historical account of the whole subject  
and complete references.)

## CHAPTER II

### THE STREPTOCOCCUS PROBLEM

THE story of the streptococci is the history of their classification. This may be divided into three stages: the morphological, the biological and the immunological. In the early days of the discovery of these organisms in wounds and septic processes by Ogston, Pasteur and Fehleisen, the wideness of their distribution was unknown and the possibility of more than one type of organism existing under a single morphological garb was scarcely considered. Later, with the appearance of the organisms in a variety of lesions, their discovery in a saprophytic state in various regions of the body, and more especially with the knowledge that the variations of their virulence for laboratory animals were extreme, it became evident that some explanation must be found for this diversity of characters, and the opinion grew up that the genus *streptococcus* included a number of varieties of very varying pathogenic ability. This contention, however, received a considerable setback from the work of Marmoreck, who upheld strongly the doctrine that there was only one species of streptococcus pathogenic for man. Those who followed him fell back upon the doctrine of differences in virulence to explain why at one time the organism might occasion an angina, at another erysipelas, and at yet another septicæmia.

The differentiation of streptococci upon biological grounds may be said to have commenced with the work of Schottmuller, who, in 1908, showed that the organisms varied in the effects which they produced upon blood cells, some causing the hæmolysis, whilst others produced a green colour, long attributed to the formation of methæmoglobin. By these reactions the varieties *S. hæmolyticus* and *S. viridans* became recognised. It seemed that herein lay the germ of the solution of the difficulty, and since it was chiefly organisms derived from pathological sources which were

hæmolytic it was thought that hæmolytic ability might be found to be definitely linked with virulence and pathogenicity. Further work, however, showed that while these properties were broadly correlated, no such exact and quantitative relationship existed.

In 1903-4 Mervyn Gordon gave a fresh impetus to the classification of streptococci by introducing the series of tests, in the main fermentative, known by his name. Andrewes and Horder, in 1906, advanced the matter very considerably in an important paper in which they detailed the results obtained by applying Gordon's tests to a large number of streptococci isolated from the most varied conditions. They divided up the genus into six large groups, excluding the pneumococci, each group centering in a specific type which was surrounded by numbers of variants, which differed from the central type in respect of one or more properties. Thus were constituted the *Salivarius*, *Pyogenes*, *Mitis*, *Fæcalis*, *Equinus* and *Anginosus* groups. The chief feature of this classification was the conception of groups rather than of definite types; its obvious inconvenience was that the groups merged by a host of intermediaries one into the other.

Another contribution to the subject, which has figured largely in subsequent work, was that of J. Howard Brown, who, in 1919, made an especially detailed study of the effects produced by streptococci upon blood agar plates, the colonies being studied in the depths of the medium as well as on the surface. By this means Brown differentiated three varieties which he designated " $\alpha$ ," " $\beta$ " and " $\gamma$ " types. The  $\alpha$  type is a green-producing streptococcus which causes a zone of partial clearing about its colonies, the pneumococcus belongs to this type. The  $\beta$  type organisms produce true hæmolysis, with a clear red halo about each colony, whilst the  $\gamma$  type fail to affect the medium in any way. Brown distinguished varieties of  $\alpha$ -hæmolysis which are now believed to be due to different degrees of peroxide production and acid hæmolysis (*vide* p. 29).

What I have called the biological phase of streptococcus investigation was characterised by many other attempts to classify streptococci, all founded more or less upon a combination of their action on blood, and sugar fermentation tests. One of the

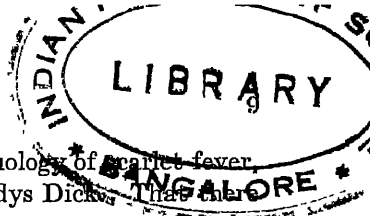
best-known examples of these, apart from that of Andrewes and Horder, was the system devised by Holman, which has had a considerable vogue. No definite and generally acceptable agreement, however, was come to as to the importance of the groups so constituted, and it was obvious that as more and more tests were introduced for the differentiation of these organisms the greater became the number of possible and actual varieties met with. Thus simplification seemed to abolish classification, elaboration only to lead to confusion; moreover, the results of such classifications were not in conformity with the pathogenic action of the organisms in the body. For instance, Holman recognised four principal types of hæmolytic streptococci. It can readily be shown from an analysis of the results obtained by this worker and others who used his or similar classifications that in such conditions as erysipelas and cellulitis any of the four types may be found as the causal organism, with approximately the same frequency of incidence in each case. Now such a finding is contrary to one of our well-established bacteriological beliefs, namely that each disease is occasioned by one species of organism. Hence any scheme of classification which showed that a single and sharply defined clinical entity could be caused by four different varieties of the same type of organism, however correct it might be upon taxonomical grounds, would be useless for practical purposes—and bacteriological classifications, in human medicine, are admittedly practical makeshifts.

The position was therefore gradually reached that whilst the biological methods of separation might be of use and informative in separating large groups of streptococci and in indicating their origin, the finer measures of classification aimed at by such methods were useless, if not actually misleading. This was more especially the case with the hæmolytic streptococci in which subdivision seemed a hopeless task.

### **SCARLET FEVER**

The view that the hæmolytic streptococci formed a united group, in which it was fruitless to seek for differences, was definitely

## SCARLET FEVER



upset by a number of workers upon the ætiology of scarlet fever, chief amongst whom were George and Gladys Dicks. That there was an intimate relationship between these organisms and the disease had long been known, and the constant occurrence of hæmolytic streptococci in the throat commented upon. Andrewes and Horder, indeed, had set up a special group of streptococci—*S. anginosus*—to include organisms isolated from this condition. There was, however, no direct proof forthcoming of any causal relationship, and the majority of more cautious bacteriologists, prior to 1923–24, had regarded this as being more than doubtful, whilst fully admitting the dominant part played by streptococci in the production of the various septic complications of the disease.

The work of the Dicks completely reorientated the view taken of this relationship and supplied the key which had previously been lacking for a fuller comprehension of the subject. To understand how this came about it is necessary to go back a little, to examine in detail certain points in the controversy, and to enquire what views were held as to the mode of action of the streptococcus in the body. The opinion had been put forward by Mosser, as early as 1902, that the scarlet fever streptococci formed an immunologically distinct group, demarcated from other hæmolytic streptococci by their serological reactions. Mosser also prepared a therapeutic serum, from strains isolated from cases of scarlatina, which was said to give favourable results in practice and specifically to agglutinate the streptococci present in this disease. This work did not gain many adherents or attract much attention, and it was left to a series of American workers, Dochez, Avery and Lancefield (1919), Tunnichff (1921), and Bliss (1922), to go over the ground again with more detail and improved methods, and to establish the general correctness of this work. Dochez, Avery and Lancefield showed that one of the major difficulties of streptococcus classification by agglutination—that of spontaneous sedimentation and clump formation—might be largely got over by growing the organisms in a special medium, in which the necessary salt concentration is obtained by a balanced phosphate mixture, with the pH adjusted to 7.4. The organisms, after

twenty-four hours' growth, only raise the acidity to  $pH\ 7.2$ , which is just on the alkaline side of that at which granule formation begins to show. In this way uniform suspensions are obtainable and the great difficulty of spontaneous granulation is avoided. It is clear, however, from their work, that a good deal of manœuvring is necessary to obtain this end and that the technique is anything but automatically successful. The organisms when grown are washed and re-suspended in the same medium at neutrality, and in this form used for agglutination which is carried out at  $55^{\circ} C.$  for one hour, the results being read immediately. Control experiments are carried out with normal serum from the same animal. Using these methods in the study of streptococci obtained from the pulmonary complications of measles, pneumonia and bronchopneumonia, and from the throats of healthy individuals in contact with such cases, Dochez, Avery and Lancefield were able to satisfy themselves that out of a total of 125 strains studied, 68 per cent. fell into one or other of four main serological groups, whilst 32 per cent. remained outside of this scheme. The general conclusion drawn was that serological types of streptococci, comparable to the better known serological types of meningococci and pneumococci, could be shown to exist, but that the results were in no way so easy to obtain or so clear-cut as with these other organisms. The same authors also carried out protection experiments, which were characterised by the production of very potent monovalent antisera and the use of strains of organisms for test purposes whose virulence had been raised to an extreme degree by passage, in order that definite and quite clear-cut results might be obtained. The serum was given twenty-four hours before the carrying out of the experiment, a condition necessary for its full protective action to be in evidence. By these means protection by small doses of serum (0.5 c.c.) was found to be afforded to mice against more than a hundred thousand lethal doses of streptococci. The results of such experiments were in complete agreement with the grouping of the organisms obtained by agglutination and, incidentally, they showed the possibility of obtaining very highly potent antisera and the sharply specific nature of these. The protection afforded to an animal by the homologous antiserum was no less

striking than its complete impotence against an antigenically different strain. The experiments which we have quoted did not include a study of streptococci from scarlet fever cases, but the methods introduced are of importance for the general solution of the problem.

With regard to the individuality of the scarlet fever streptococci, Tunnichff (1920), in a somewhat restricted series of experiments, was able to show by agglutination and opsonic methods that a monovalent sheep serum, prepared from a streptococcus of scarlatinal origin, specifically affected other scarlatinal streptococci, which thus comprised an immunological group. Protection tests with the same serum gave confirmatory results. Bliss, in his early experiments (1920), found that monovalent sera prepared from scarlatinal strains of streptococci agglutinated a large majority of organisms isolated from this disease, each strain being agglutinated by several of the monovalent sera. Sera prepared from hæmolytic streptococci found in other conditions, including those representative of the types defined by Dochez, Avery and Lancefield, generally failed to agglutinate the scarlet fever organisms.

Bliss, in a later study (1922), with an additional batch of scarlet fever streptococci, confirmed his former results, whilst subsequently "puerperal scarlet fever," "wound scarlet fever," and "burn scarlet fever," conditions which had long been considered to be streptococcal infections, but of somewhat dubious relationship to idiopathic scarlatina, were shown to yield streptococci belonging to the same immunological group (Stevens and Dochez, 1924). Bliss also found, as Gordon did, that a scarlet fever streptococcus removed from homologous serum the specific agglutinin for that strain, and also for other scarlet fever streptococci. On the other hand, streptococci from non-scarlet sources did not absorb these agglutinins. The converse held good, the scarlet fever streptococci failing to absorb agglutinins present in sera prepared from organisms isolated from other types of infection.

A further point which was cleared up by Stevens and Dochez (1924) was that the streptococci obtained from cases of scarlet

fever in widely separated countries similarly fell into this homologous group.

M Gordon, in this country in 1921, published a paper in which he detailed the results he had obtained by working on similar lines. Taking an unselected group of sixty-eight streptococci, derived from various pyogenic conditions, he prepared an agglutinating serum from one of these which, on subsequent trial by absorption methods, proved specific for sixty-six of the organisms. This serologically homogeneous group he classified as Group I (Gordon). The two remaining types, which failed to absorb the specific Group I. agglutinins, proved on further examination to be serologically independent, and from them he prepared further agglutinating sera designated Groups II. and III. The examination of a small number of streptococci from puerperal sepsis (10) showed these all to belong to Group I., whilst thirty-one out of thirty-six strains from respiratory infections also fell into the same group. Gordon therefore concluded that the pyogenic streptococci in the more ordinary septic conditions belonged very largely to one and the same immunological group. Turning to the scarlatinal streptococci, he examined eighteen strains and found that sixteen of these were serologically homologous, but that none of these corresponded to his Group I.—the common pyogenic variety. His type III. organism, however, absorbed the agglutinins from sera prepared by the injection of these scarlatinal strains, which in consequence fell for classification with this group (Group III.) Up to the present Gordon's Group II. appears to be a trivial one, of doubtful importance for the larger aspects of the subject.

The findings which have resulted from all these agglutination tests, which we have reviewed in their broadest aspects, have not received unqualified confirmation. J Smith (1926), for example, found the scarlatinal group to be anything but homogeneous and distinguished certain sub-groups within it, and this has been the experience of most English workers. Stevens and Dochez (1926) also admit this to be the case, but contend that the constituent sub-groups of the scarlet fever streptococci are more closely related to one another than they are to organisms derived from other pathological conditions. Whilst, therefore, the results

which we have noticed may be understood to hold as true when they are viewed as a whole, it must be remembered that the boundaries of the group are ill-demarcated and, further, that striking individual exceptions occur with certain strains. The combined mass of evidence, however, cannot fail to be regarded as impressive.

Thus by the investigations of the workers on type specificity, the study of the hæmolytic streptococci was advanced to a point at which it became evident that distinct immunological strains existed in the streptococcus family, and that one broad group of these appeared definitely to be associated with scarlet fever.

**The Toxic Products of the Scarletinal Streptococci.**—Another side of the problem which required illuminating was the method by which the streptococcus produced its results. It had long been a settled belief of pre-war bacteriology that, apart from the diphtheria bacillus, the tetanus bacillus, and *B. botulinus*, the pathogenic micro-organisms were in the main active by means of endotoxins. Attempts to produce soluble toxins from streptococci had notoriously failed. Products were obtained, notably by Marmorek and others, which gave rise to disturbances in the health of laboratory animals, and even to their death, but to produce any noteworthy results these had to be injected in relatively enormous quantities and in lesser doses were found to be inert. The view therefore gained tacit acceptance that the action of the organisms in the body was an endotoxic one, although this received little confirmation from actual experiments, lysates of streptococcus body-substance being non-toxic.

In a disease such as scarlet fever, where in uncomplicated cases the organisms remain localised in the throat, the general symptoms and rash are evidently toxic phenomena, and the total failure to demonstrate even the possibility of this being a result of the streptococcus invasion was one of the weightiest of the arguments existing against such a view. The link missing from the chain of evidence was hinted at by the discoveries of Schultz and Charlton, and was finally disclosed by the Dicks. What is now generally known as the Schultz-Charlton phenomenon was noted by those observers in 1918, and consists in the fact that injections of the

serum of convalescents from scarlet fever, and in some cases of normal human serum, cause local blanching of the scarlatinal exanthem in the area about the injection. This was taken to denote the existence of an antitoxic substance in the blood during the later phases of the disease and in convalescence—a substance present sometimes in normal serum, which would neutralise the poison responsible for the rash.

In 1923, and the years following, George and Gladys Dick, of Chicago, carried out some experiments which in our present state of knowledge seem crucial ones. They took up, firstly, the undecided question of the possibility of experimentally transmitting scarlet fever from man to man. Their volunteers were carefully selected, so as to exclude as far as possible the ever present fallacy of spontaneous infection, and these were subjected to swabbing of the throat with cultures of a hæmolytic streptococcus which had been obtained from a secondary abscess on the finger of a nurse suffering from a typical form of the disease. One subject out of five experimented upon developed an attack of scarlet fever with rash and desquamation, one had a sore throat and the others remained well. At the same time the throats of five other volunteers were swabbed with Berkefeld V. filtrates from the same cultures; none of these contracted the disease. Eleven to thirteen days later these controls were again inoculated, this time by swabbing the tonsils with the live cultures. One subject developed a typical attack of scarlet fever. The Dicks therefore drew the inference that a *contagium vivum*, in the form of the streptococcus, was present and responsible for the disease. The experiments with filtered cultures excluded the possibility of a filter-passing contaminant being responsible for their results.

The organism used in the above experiments was a mannite fermenter, which is not the predominant type of streptococcus in the scarlatinal angina. A second series of inoculations was consequently carried out upon two further volunteers, with a non-mannite fermenting streptococcus. One of these volunteers was negative to the skin test (Dick test, p. 15), the other positive. The last-named contracted typical scarlet fever, and George and Gladys Dick accordingly claimed to have fulfilled Koch's postulates

and affirmed the ætiological rôle of these streptococci in scarlet fever.

The next step taken by these observers was the direct observation of the toxic effects of soluble substances produced by the streptococci upon man—the demonstration of the previously undiscovered extracellular toxin. This they succeeded in doing by utilising as a source of toxin the condensation water from blood-agar slopes upon which the streptococci had been grown. They were able to show that diluted, sterile, Berkefeld W filtrates of this substance, when injected intracutaneously in man, in the way employed in the Schick test, produced in about 40 per cent. of persons who had no history of scarlet fever a typical reaction, consisting in the production within eighteen to thirty-six hours of a circumscribed erythematous area, of the size of a half-penny or larger, which becomes swollen, cedematous and painful. It was thus, for the first time, demonstrated that the hæmolytic streptococci of scarlet fever produced a soluble toxic substance giving a definite skin reaction in man (Dick test). The Dick toxin failed to give any reaction in convalescents from scarlet fever. Injected in larger quantity intramuscularly into susceptible persons it provoked a sharp illness, sometimes associated with a scarlatiniform rash. Upon recovery from this the person was found to react negatively to the Dick test. The toxin, moreover, failed entirely to produce a like effect upon animals, and undoubtedly the postponement of its discovery is attributable to the previous neglect of direct experiments upon man. It may therefore in one sense be regarded as a by-product of the Schick test, which provided for the first time an innocuous method of testing a bacterial toxin upon the human subject.

**Nature of the Dick Toxin.**—This is unlike most of the soluble bacterial toxins in that it is a relatively thermostable substance, being capable of withstanding boiling for a variable time without undergoing destruction. Control materials are therefore difficult to prepare, and pseudo-reactions to the skin test are not altogether easy to eliminate. The substance is also non-toxic to the ordinary laboratory animals. Since the first demonstration of this toxin methods have been introduced of bulk production,

which yield a much larger quantity of the substance than the original method which we have described here. The toxin is standardised upon the human subject, its dilution being adjusted to give an equivalent result to that of a "standard" toxin.

The relationship of the Dick reaction to the disease has been extensively studied in many countries since its discovery. Although some points are not yet entirely worked out, there is a general agreement that the test is positive in the earliest stages of an attack of scarlet fever; it becomes negative towards the middle period of the disease, and is very generally negative in convalescence. Exceptions have been noted.

. . . . .

The chain of evidence, as we have set it out, is now nearing completion. The broad serological uniformity of the organisms has been dealt with; the production of a toxin capable of setting up a skin reaction in susceptible persons has been demonstrated, as well as has the disappearance of this susceptibility in convalescence from the disease. The chief remaining question is that of the means whereby such a neutralisation is brought about and the possibility of an antitoxin being produced.

It was found, in the first instance, that the serum of convalescents, capable of bringing about the Schultz-Charlton effect, had the power of neutralising the Dick toxin *in vitro*, whilst injections of a larger bulk of the same serum intramuscularly had the effect of rendering the test negative in persons who previously had reacted positively. It therefore appeared evident that ~~the~~ quality in a serum which enabled it to abolish the scarlet fever rash was associated with an ability to neutralise the Dick toxin, suggesting, therefore, that one and the same toxic factor was present in both instances. This suggestion would be further strengthened if an artificial antitoxic serum could be produced by injections of the Dick toxin which would produce the Schultz-Charlton effect, and be of therapeutic value in treating the disease. This has been done, scarlatinal antisera having been produced by a number of workers, including the Dicks, who utilised their filtered toxin for injection into horses, and by Dochez, who

employs a different method growing the streptococci in masses of agar which have previously been injected into the subcutaneous tissue of horses, upon the presumption that under these conditions toxin will be continuously formed and by absorption produce a specific antigenic effect. Such sera will produce the Schultz-Charlton reaction in cases of scarlatina, and will actively neutralise the Dick toxin, so that the presumption now becomes very strong that one and the same toxic agent is responsible both for the Dick reaction and the scarlatinal rash, and that this is the specific product of the scarlatinal group of streptococci

~ The evidence for the streptococcal origin of scarlet fever may be summarised as follows —

- (1) The constant finding of hæmolytic streptococci in the throat in the disease and in the lesions which complicate it.
- (2) The possibility of broadly grouping these streptococci into certain distinct serological types.
- (3) The production of scarlet fever in certain instances by the introduction of cultures of these organisms into the throats of healthy individuals
- (4) The presence, in susceptible individuals, of a skin reaction in response to the soluble toxins of these cocci and its absence in convalescents from the disease.
- (5) The neutralisation of this toxin by the serum of scarlatinal convalescents.
- (6) The production of an antiserum, by the immunisation of animals against this toxin, which will produce the Schultz-Charlton effect in cases of scarlet fever.
- (7) The favourable therapeutic results obtained with this antiserum.

**Antistreptococcus Scarlet Fever Serum.**—The standardisation of this serum is rendered difficult by the fact that the human subject is the only known reactor to the scarlatinal toxin. As at present carried out, the method of standardisation depends upon the neutralisation of the toxin from the point of view of its effect in causing the intradermal (Dick) reaction in man. Wadsworth states

that he is able to carry out a preliminary standardisation of toxin upon the goat, but the experience of others in this direction has not been satisfactory. Parish and Okell (1927) have had some success in preliminary titrations in the rabbit, which enable them to roughly classify sera according to their antitoxin content. In any case the final adjustments are made upon man and the antitoxin content of the serum expressed by the number of "skin test doses" which 1.0 c.c. of the antitoxin will neutralise. The official United States standard requires that 1.0 c.c. of serum shall neutralise at least 1,000 skin test doses of the toxin. Recently there has been an inclination to standardise serum by means of the "blanching dose" (Schultz-Charlton), rather than by the skin neutralisation method (O'Brien, 1925).

The scarlatinal antitoxin, like other bacterial antitoxins, is contained in the globulin fraction of the serum, which may therefore be concentrated upon lines familiar in the manufacture of other antitoxic sera. Good results in its clinical use have been reported both in this country and in the United States. It is necessary that the serum should be given early in the disease, as in the later stages its action appears slight or *nil*. In this country its use has been confined mainly to the severer cases, and its effect is often remarkable in reducing temperature and relieving symptoms. The general opinion is that it has little effect upon the incidence of the septic complications. Most of the commercial sera in use are antitoxic, and the experiments of Parish and Okell have demonstrated that such sera possess a definitely protective action against the acute septicæmic phase of a streptococcal infection. Park and Williams have suggested, and put into practice, the production of a serum of a dual nature, partly antitoxic and partly antibacterial.

Anti-scarlatinal serum is also said to have a beneficial protective action and to be capable of utilisation for the production of passive immunity and the prevention of the disease in contacts.

**Active Immunisation.**—Attempts are in process of being made to actively immunise children, and nurses in fever hospitals, against scarlet fever upon the lines successfully followed in diphtheria. Injections of small quantities of the Dick toxin (*e.g.*, 1,000 minimal

skin doses) have the effect of converting a positively reacting subject into a negatively reacting one, which may be taken to indicate a satisfactory degree of immunity. This immunity, as evidenced by the skin test, is not of very long duration, and it is known that an attack of scarlet fever does not result in a permanently negative condition towards this test.

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## ERYSIPELAS

Tunnichliff, in 1920, duplicated with erysipelas the work which, as we have previously noticed (p. 11), she carried out on the specificity of scarlet fever streptococci. Using a single serum, obtained by immunising a sheep, she found that this agglutinated ten out of thirteen erysipelas strains of streptococci, but failed to agglutinate any of the twenty-four control strains from other sources. The study of the thermostable opsonins and absorption tests with the monovalent agglutinating serum gave similar results, all pointing to a serological specificity of the erysipelas strains. This work cannot be regarded as giving more than an indication in this direction, since the agglutinations were obtained at very varying dilutions and only a single serum was used.

Birkhaugh (1925) produced potent agglutinating sera by what he calls "intracutaneous" injection of agar, inoculated with live cultures of streptococci from cases of erysipelas, into rabbits (it is evident from the context that these injections were intramuscular), following the technique of Dochez. Using unheated sera, he found thirty-one out of thirty-four erysipelas strains to agglutinate with the eight sera so prepared, whilst the agglutination of hæmolytic streptococci from other sources was infrequent and irregular. By absorption experiments it was found that the erysipelas strains removed the specific agglutinins from these sera. In later experiments (1925) he confirmed these findings by protection tests carried out in rabbits, and claimed that no crossed immunity with scarlatinal strains existed. At the annual meeting of the British Medical Association, in 1926, Birkhaugh amplified these findings by reference to the results obtained in the treatment of erysipelas with an antiserum prepared from his specific strains. The clinical effect of this, which was given in doses of about 100 c.c. of the unconcentrated serum, suggested that it had a striking therapeutic action, a single dose being usually followed by

a critical drop in temperature and an immediate and permanent amelioration of all symptoms. Such results were only obtained when the serum was administered early in the disease.

Singer and Kaplan (1926) prepared a toxin from erysipelas strains of streptococci, which gave a skin reaction similar to the Dick reaction. This was neutralised by the serum of convalescents and also by an immune serum which was obtained by injecting two human volunteers with the toxic filtrates, but they did not find the same degree of distinction between scarlatinal and erysipelas streptococci, on the basis of this reaction, that Birkhaugh claimed.

Eagles (1926) found that puerperal and erysipelas strains were toxigenic in the same way as scarlatinal strains are, and that although, when rabbits were employed as the source of antitoxic sera, there was little crossed neutralisation between these and the different toxins, yet anti-scarlatinal serum (commercial) neutralised all of the toxins, irrespective of their source.

Other workers have also failed to obtain anything like the same sharply-cut results as Birkhaugh ; and Stevens and Dochez (1926), in a very careful study, failed to show any sharp line of cleavage between scarlatinal and erysipelas streptococci. They conclude that "Erysipelas strains form a closely-related group of hæmolytic streptococci. Scarlatinal strains form an equally compact group. The two groups are related antigenically, but less closely related than the strains within the groups. These groups are related to pyogenic strains, but less closely than they are related to each other."

### PUERPERAL SEPSIS

We have already noted that Gordon (1921) found the ten puerperal strains of streptococci examined by him to be identical with the pyogenic forms with which he worked. Eagles (1924), in a restricted series of agglutination experiments, obtained some indication of an immunological distinction between puerperal strains and those from erysipelas and common septic conditions. Lash and Kaplan (1926), upon lines which are now thoroughly familiar, succeeded in isolating a toxic filtrate from growths of

puerperal streptococci which gave a skin reaction similar to that first described in scarlet fever. Using higher dilutions of this toxin, to obtain information as to the presence or absence of immunity towards it, they met with the somewhat curious result that whilst only a very low proportion of non-pregnant healthy women gave the reaction, and healthy pregnant women showed approximately the same incidence of positive results, women in the puerperium, suffering from minor septic complications, gave a much higher proportion of positive tests, and apparently healthy women, tested shortly after labour, a maximum incidence of these. If this should prove to be the case it would seem to show that the normal resistance of the pregnant woman breaks down after labour, leaving her in an unusually susceptible condition. In further investigations these workers have shown that their toxin is heat-labile, in this respect differing from the Dick toxin, and is capable of inducing an antitoxin response on injection into rabbits.

Very recently Burt-White has tested the sensitivity of a series of a hundred pregnant women to the skin reaction with a Dick toxin prepared by Okell. He found that twenty-seven of these gave a positive test, and amongst them eight cases of sepsis occurred. There were seventy-three women in whom the reaction was negative, and amongst these were two cases of puerperal sepsis, but neither of them was attributed to streptococcus infection. If these observations should be extended, and if it should be proven that skin sensitiveness to streptococcus toxin indicates a tendency to puerperal sepsis which is a dominating factor in determining this, then a really valuable means of prophylaxis may be in our hands. It should be possible to select the subjects who are threatened with this complication and immunise them in the course of their pregnancy by the same methods as have been successfully employed in the protection of nurses against scarlatina in the fever hospitals.

An anaerobic organism, hitherto unreported in septic puerperal conditions, has been discovered by Harris and Brown (1927) in five cases of sepsis complicating the puerperium. This is a thin, Gram-negative, non-sporing, non-hæmolytic, non-motile bacillus,

which closely resembles *Actinomyces necrophorus*, familiar to veterinarians in necrotic lesions in a number of domestic animals. Harris and Brown do not consider their organism highly pathogenic for the human subject, but suggest that it may play a definite part in some cases of puerperal infection.

### STRANGLES (*S. Equi*)

In a series of papers (1922-1925) Brocq-Rousseau, Forgeot and Urban claim that the hæmolytic streptococci which they isolated from horses suffering from this disease showed uniform and distinctive serological properties. Utilising the complement-fixation test, they state that practically without exception the strangles strains absorbed complement in the presence of a serum prepared from one of them, whilst streptococci from other equine diseases, from other animal sources, and from human sources did not do so. They have further prepared an antiserum from their strangles streptococci, from the use of which they report favourable results in aborting the disease in its early phase and, in more advanced cases, in reducing temperature and preventing the suppurative evolution of the lesions.

### SUMMARY UPON THE SEROLOGICAL GROUPING OF THE HÆMOLYTIC STREPTOCOCCI

It is a natural characteristic of research workers in all countries that they publish their positive findings but often relegate their negative ones to oblivion. In the case of the agglutination grouping of the streptococci this statement holds strongly true. Summarised, as general conclusions must be in a review of this sort, the results obtained by Dochez, Gordon, Bliss and others appear clear-cut and relatively simple in obtaining. Nothing is really further from the truth. The agglutination of the streptococci is a business hedged about with difficulties, demanding much patience and careful manoeuvring of technique if results are to be obtained at all; and these difficulties are many times multiplied when absorption methods are used. There are, moreover, in almost every series of chronicled results instances in which organisms

have been found which will not fit into the described scheme or which under no circumstances can be obtained in a condition suitable for agglutination. Further, in the work of certain investigators (James (1926), Griffiths (1927), Smith (1927) ), sub-groups have been found to occur within the main groups which encompass the majority of serological strains. McLaughlan and Mackie (1928), go so far as to deny the existence of a specific scarlatinal group. It would appear, however, to be now a fairly well established finding that a majority of the streptococci from cases of scarlet fever fall into a certain broad serological group or series of groups. This has been the finding of many workers in different countries, and though others have not been able to confirm these results, the positive findings appear sufficiently well established to permit their acceptance.

With regard to serological groups other than the scarlatinal, the matter here is much less settled. Although sharp differentiation has been found by Birkhaugh between the erysipelas and other strains, this lacks confirmation, and Stevens and Dochez report a fairly close association between these organisms and those present in scarlet fever. In yet other conditions—general surgical septic processes, in which Gordon distinguishes his Group I. (pyogenes) strain, and puerperal sepsis in which observations are as yet insufficient for any conclusions to be drawn—the relationship of the various forms encountered still remains an open question.

There is also the further problem of the meaning and permanence of the distinctions which have been made. As regards the first point, opinion generally leans towards the existence of a very complex antigenic substance in the streptococci, some portions of which are broadly distributed, others specific only to certain groups. Stevens and Dochez illustrate this view by suggesting that if the antigenic components of the scarlatinal strains be represented by the letters a b c d e f g h, then those of the erysipelas strains may be e f g h i j k. The chance agglutination of strains from other sources by sera in the main specific for one or other of these groups would be due to the presence of some of these components in common.

The permanence of the immunological groups is as yet unsettled.

In certain quarters it seems assumed that the properties which render streptococcal groups antigenically distinct are permanent or semi-permanent, as is generally the case with the bacterial species with which the earlier work on agglutination was carried out. There are indications, however, that in the streptococci, many of which are susceptible to variations in their other biological properties, this may not be the case. Eagles has put forward such a suggestion to account for the disappearance of the scarlatinal group from the throat of patients in convalescence and, in another place, hints that the occurrence of serological varieties may be simply the effect of environment. The French workers on strangles categorically state that their strains lose their specific qualities on passage through the body of another animal, and also after cultivation on certain artificial media, reverting to an undistinguished "streptocoque de passage"—a conception fairly common amongst French bacteriologists. Whilst leaving this question open it may be well to recall that during the biological period of streptococcus classification, as I have termed it, when observers were mainly concerned with sugar reactions, the general consensus of opinion reached was that these tests served to point out with a fairly reliable degree of accuracy what had been the immediate previous environment of the organisms, and this only when they were tested shortly after isolation. Long experimentation and cultivation might rob them of their distinctive properties.

The suggestion has been put forward by F. W. Andrewes that many of the difficulties in working upon the agglutinative reactions of this group may be due to the presence of specific and non-specific strains, akin to those described by him in the salmonella group (p. 46). In a verbal communication upon this subject to the Pathological Society of Great Britain and Ireland, Andrewes stated that his incompleted experiments pointed to the conclusion that the hæmolytic streptococci could be divided into "rough" and "smooth" strains, the rough strains being the specific ones and yielding specific agglutinins, the smooth strains being non-specific and giving rise to agglutinating sera of a group character. This suggestion, if borne out by further work, may greatly simplify the problem.

There is yet another aspect of the modern conception of group specificity amongst the streptococci which requires consideration. At the time of the discovery of the toxigenic properties of the scarlet fever streptococci by Gladys and George Dick, and for a considerable period thereafter, it was tacitly assumed that this was a distinctive possession of the scarlatinal strains and that the toxin was a specific one. Birkhaugh supported this view by contending that the toxin produced by his erysipelas strains was specific for erysipelas, whilst Lash and Kaplan made the same claim, *mutatis mutandis*, for the puerperal organisms. It may be recalled, however, that Singer and Kaplan, in a small experiment, found that an antiserum active against an erysipelas skin toxin was also effective against a scarlatinal toxin. Eagles, also, found that although with monovalent rabbit sera some specificity between the toxins and antitoxins of puerperal and erysipelas strains was noticeable, this disappeared when commercial scarlatinal antitoxic serum was used, this neutralising all of the streptococcus skin toxins irrespective of their origin.

The general tendency to be observed in this work is a move from the "specific" position, at first taken up by most bacteriologists after the discoveries of the Dicks had become known, towards one in which the differences are realised to be more quantitative than qualitative. This same movement is discernible in the results of the workers upon agglutination, and has already been referred to. Parish and Okell have adduced a considerable amount of direct evidence in this respect. They had previously shown that an antitoxic streptococcal serum has a definite protective effect against the acute phase of a streptococcus septicæmia in rabbits, and in their later experiments (1928) they found that this effect was produced by an anti-scarlatinal serum, not only against homologous organisms but against hæmolytic streptococci coming from diverse sources, such as erysipelas, puerperal sepsis, endocarditis, surgical septic conditions, etc. They also found the converse to be true, and that sera prepared from filtrates of scarlet fever, erysipelas, puerperal or pyogenic streptococci, all of which produced the Dick toxin to some extent, were protective against infection with scarlet fever streptococci.

There was, however, a quantitative difference, the scarlet fever antitoxin proving more actively protective than those coming from the other sources.

The general conclusions which these workers have arrived at are that the protective action of streptococcus antitoxic sera is the same in all cases. the effect of a single antitoxin. The hæmolytic streptococci owe their toxic effects to one and the same toxic substance, the clinical differences in these depending upon the amount of toxin which the individual strain of organism is capable of generating and, of course, upon conditions in the human substrate. This position is essentially that of Marmorek (1895) and the unicists. It is also practically that taken up by French bacteriologists with regard to the question of pneumococcal types.

Regarded from this point of view, the scarlet fever streptococcus is merely an especially active toxigenic variety, and therefore an antitoxin prepared from it is especially likely to contain a high concentration of the specific anti-substances, a suggestion which the experiments of Okell and Parish bear out. It must be pointed out, however, that their results are in direct conflict with those of Dochez, Avery and Lancefield (1919), who found a high degree of strain specificity in their protection experiments with hæmolytic streptococci. Their antisera, however, were obtained from sheep, rabbits and dogs, whilst those of Parish and Okell were derived from horses, which, as Eagles has observed, may account for certain differences in results. It does not follow that an identical mechanism was at work in the two cases, and indeed the experiments of the English workers indicate that the protection afforded by antitoxic serum is a very complex affair, an adequate prevention of septicæmic death not being correlated with immunity from localised subacute or chronic streptococcal lesions.

A logical outcome of the view developed by Okell and Parish would be the reversion to the use of monovalent anti-streptococcal serum for therapeutic purposes, which could be produced from any streptococcus, irrespective of its origin, which proved to be an active toxin producer. Such a serum should be effective both against scarlet fever and any other toxic or septicæmic streptococcal infection. A useful scope for its employment, which is

suggested, is in the prophylactic treatment of accidental surgical or post-mortem infections. It has also been suggested—and there is a good deal of general agreement upon this point—that the relative impotence of many of the older anti-streptococcus sera, which resulted in the abandonment of this therapeutic weapon, was due to their being prepared by the injection of bacterial bodies, and being in consequence practically devoid of the essential antitoxic properties.

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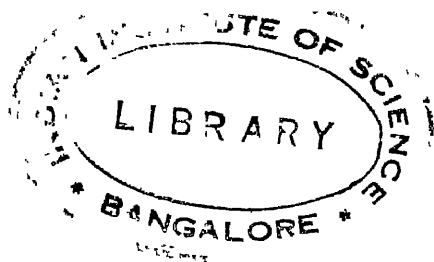
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## THE NON-HÆMOLYTIC STREPTOCOCCI

The non-hæmolytic members of the streptococcus genus are still classified upon biological lines. By their action on blood the large viridans group may be split off, and from the remnant the enterococcus group may be separated by certain fairly characteristic tests.

**The Viridans Streptococci.**—The habit has grown up of designating all non-hæmolytic strains as “viridans.” This would seem unfortunate, and it would be better if this term were reserved only for those organisms which produce a definite green colour upon blood agar. This reaction has long been held to result from the transformation of hæmoglobin into methæmoglobin, but the work of McLeod and Gordon (1928) has shown that the colour change is actually due to the production of peroxide of hydrogen by the organisms. It is the presence of this substance also which gives the yellow discoloration on Warren Crowe’s and other “chocolate” media. This is not to be taken to mean that no methæmoglobin formation occurs, since most viridans streptococci oxidise hæmoglobin in this way, but to indicate that two processes are at work in the production of this reaction which are not necessarily correlated.

The chief pathological processes in which these streptococci are of interest are ulcerative endocarditis and rheumatic fever. In the former condition the experience of all workers is that these organisms, and those of the inert group, are the most frequent invaders in the subacute type of this disease so common at the present time. Attempts have been made to first still remark of serological grouping amongst the viridans and that Swift and to that described in the hæmolytic streptococci protective action result, and it is practically unanimously agreed that *S. viridans* form a heterogeneous group just as the pneumococci, not great, evidence

The heavy mortality provoked by the

group of organisms, when they lodge upon the heart's valves, causes some surprise, especially when it is known that a high concentration of antibodies often exists in the patient's blood. The suggestion is made by Wright (1925) that the physical circumstances of the organisms' location, and their sheltered situation from leucocyte action, may account for their obstinate persistence in this condition. This is not the whole story, however, for Parish and Okell found that in animals which had recovered from septicæmia, owing to treatment with anti-streptococcus serum, a second phase of infection supervened, with the production of local lesions in joints and serous sacs, which was not prevented or affected by the presence of antibodies.

With regard to the still vexed question of rheumatism, little advance has been made. Streptococci of the viridans or inert groups continue to be isolated by some observers from blood and synovia, and are as persistently absent in the experience of others. The old observations of Shaw (1904), Beattie (1904-12) and others, that injected intravenously these organisms *can* cause arthritis, often non-suppurative, and endocarditis, have been repeated again and again, but the sum of our actual advance in knowledge is slight. In fact many of the claims to have produced the Aschoff bodies in experimental animals, which are becoming increasingly to be regarded as the essential lesion in acute rheumatism, seem untenable in the light of later criticism. Topley and Weir (1921) went over the ground again and, in a rather damaging paper for the conception of a *S. rheumaticus* as a special entity, showed that similar lesions in experimental animals are produced by streptococci not only from rheumatic but also from many other sources. They did not find in any cases typical Aschoff bodies. In a guarded way they support the view that the disease may be caused by streptococci, which have either an attenuated infective capacity and peculiar properties of localisation as Rosenow point out that if rheumatic fever be not due to organisms alone are the sole bacterial claimants, two at the same time—an *a priori* argument

Miller (1924) investigated claims made by De Vecchi (1912) and Natali (1923) that the disease was due to a filter-passing virus and obtained results which were almost wholly negative. Though some formations resembling Aschoff bodies were found in certain of his animals, similar structures were seen in the controls. This worker in a subsequent paper rendered a considerable service to research in this field by demonstrating that some 60 per cent. of laboratory rabbits showed myocardial lesions which might be construed by the enthusiastic as being Aschoff bodies ! Such a finding is worth bearing in mind in evaluating the reports of certain experimental results in this disease. It must of course be recognised that the non-appearance of Aschoff bodies in experimental animals is not a refuting argument of very great weight. It may well be that the reaction of the animal to the causal agent differs both in kind and degree from that which in the human subject finds expression in these formations.

Birkhaugh (1927) describes a non-*viridans*, inulin-fermenting streptococcus, as present in the throat and other situations in rheumatic fever, from which he claims to have isolated a soluble toxin. Small (1923) also describes a somewhat similar organism, which is pathogenic for the rabbit, from which he claims to have produced a therapeutic antiserum.

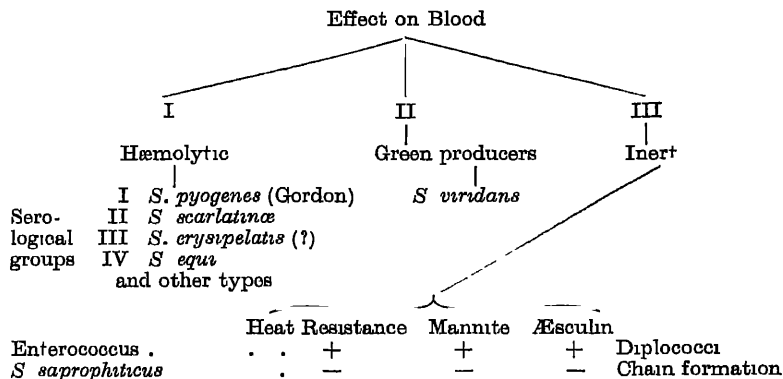
On the whole the general case for the streptococcus as the immediate cause of acute rheumatism does not seem strengthened. The more attractive view, if the streptococcus theory be accepted, is that the rheumatic manifestations are toxic ones, and not due to the actual presence of the organisms in the tissues locally. The possibilities and frequency of saprophytic organisms, such as the non-hæmolytic streptococci, gaining access to the body, and of their localisation in any *locus minoris resistentiæ*, is a matter on which much more light is desirable. The old criticism of the striking effect of salicylates still remains a forcible one, though it is of interest to find that Swift and Boots (1923), who made observations on the protective action of this substance in rabbits in which experimental *S. viridans* arthritis was induced, found definite, though not great, evidence of a protective action of the drug.

**THE GROUP OF STREPTOCOCCI INERT TOWARDS BLOOD**

The majority of organisms of this type are to be found in the throat, mouth and intestine. They form a considerable group, including the *S. salivarius*, *S. mitis*, and *S. faecalis* of the older writers. Amongst organisms included in these three types are a certain number of green-producers which may be put into the viridans class. There remain, however, a large group, of which some are chain-forming organisms whilst others grow generally as diplococci or at the most form short and irregular chains. The work of the author (Dible, 1921) separated out from amongst these a well-defined class previously partially described by French and German writers as the *Enterococcus* and *M. ovalis* respectively. The salient features of these organisms, for which the term enterococcus seems now fairly established, is that they produce a uniform haze when grown in broth, forming as a rule diplococci. A large proportion of them ferment mannite and they are highly heat-resistant; broth cultures withstanding an exposure of twenty to thirty minutes, or more, to 60° C, whereas five to ten minutes at the same temperature generally serves to kill off the hæmolytic streptococci. A further property of this group has been recently added (Meyer, 1926), that of splitting æsculin. This substance was introduced into bacteriological work by Harrison and Van der Leek in 1907 as a means of differentiating "*B. coli*" from similar organisms. Its decomposition yields a hydrolytic product which gives an ink-like compound with iron salts if these are incorporated in the medium. The æsculin reaction has been found by Weatherall and the author to be given by a large proportion of enterococci, but it is more liable to be positive with pathogenic streptococci than the thermo-resistance test is. This group of organisms shows no serological homogeneity. Bagger (1926) has tested this, and concludes that the organisms are strain-specific and that the reaction is therefore useless. In general their pathogenicity for laboratory animals is low, though there is some evidence that strains isolated from appendicitis, or from the fæces in typhoid and other pathological conditions, have a certain degree of virulence. Many of the milk streptococci,

*S. lactis*, are similar to if not identical with the enterococcus, though, as is well known, a great variety of streptococci, some of them hæmolytic, are to be found in milk.

The present position of the streptococcus problem may be summarised by the following scheme of classification.



N B—The term “saprophiticus” is not used here in the narrow sense, but to indicate the large body of streptococci, met with chiefly as saprophytes in human pathology, which do not fall within this classification, and in which distinctions are of unknown value, *e g*, various types of *S. salivarius*, *S. mitis*, *S. equinus*, &c

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## CHAPTER III

### BACTERIAL VARIATION

PERHAPS no subject in the whole of bacteriological science has had so chequered a career as that of bacterial variation in regard to its possibility, existence and scope. Regarded from the viewpoint of general biology, no kind of living organism would appear so likely to exhibit the phenomenon as these lowly forms, whose rate of multiplication in favourable circumstances can be so colossal. When hundreds of generations can be passed through overnight the opportunities for environmental conditions to modify the organisms appear limitless, and it would seem to be a necessity that modifications should occur if such influences exert any effect at all upon the living and multiplying cell.

Curious though it may seem, it is nevertheless a fact that such possibilities have been in general rather frowned upon by the great majority of practising bacteriologists, largely, one assumes, because by excluding their possibility a threatening chaos is kept in the background and a great economy in mental effort is achieved. It is the penalty of most medical bacteriologists that they are chained to the utilitarian side of their subject and have little opportunity for studying its more speculative aspects. This is indeed a pity, and undoubtedly it has hampered progress in general knowledge of bacterial life.

In the early days of microbiology a number of workers, Naegeli chief amongst them, taught that variation amongst microbes was the rule, and that transformations from bacteria to cocci or to spullary forms were common occurrences. The exact work and outlook of Koch and his school not only demonstrated the erroneous nature of such speculations, but rigorously suppressed unorthodoxy of this nature and, by carrying the matter to the other extreme, established for a long period the viewpoint that

bacteria of each species were all alike, unchanging, and rigidly defined by a certain set of standards. Hence the classic text-book descriptions which the unfortunate student endeavours, with varying degrees of success, to commit to memory

The inconvenient occurrence of changes in colony appearance and in microbic form, notorious for example in *B. diphtheriae* and *B. pestis*, were labelled "involution changes" and consigned to oblivion with this self-satisfying cognomen. The modern bacteriologist, confronted with a confused array of "roughs and smooths," with H's and O's, may well wish they had remained there!

The Germans were good schoolmasters and, like all good schoolmasters, dogmatic. The teaching we have just reviewed no doubt saved bacteriology from awful chaos and saw it through the years of adolescence, enabling it to develop on the sound utilitarian lines which medical progress has demanded. Nevertheless with advancing knowledge there comes a period when the old dogmas of school days are found wanting, though their utility remains undoubted, and when the opening of further fields necessitates a change, or at least a modification, of view.

We have perhaps exaggerated the bacteriologist's belief in the fixity of his species, for no one can practise this subject and long remain bound to that belief. Exceptions have been known, and well known, since the earliest days of the science, and yet with a curious degree of inconsistency they have, by their very respectability, hardly been held to impugn this doctrine. The most classical of these is probably that achieved by Pasteur in 1881, who, by exposing anthrax bacilli to a high temperature, produced a permanent modification of their characters obtaining a race which was both non-sporing and of diminished virulence. This discovery was preceded by the establishment of a similar modification of virulence, also produced by altered environmental conditions, in the bacillus of fowl cholera; but the result with anthrax is probably the best-known, though not the earliest, instance of a mutational effect being produced at will in a pathogenic bacterium.

Another striking and early recognised example of bacterial variation is that displayed by *B. coli mutabile*, studied by Neisser

and by Massini (1906-7). Colonies of this non-lactose-fermenting organism developing on lactose-fuschin-agar plates produced, after two or three days, papillæ which were red in colour. Re-plating from these gave a mixture of red and white colonies, and on re-plating from the red colonies the lactose-fermenting mutant bred true. This type of experiment was extended to other bacterial species by various workers, amongst whom was Penfold who succeeded in producing a dulcitate-fermenting mutant of *B. typhosus*. The variability of another group of micro-organisms—the streptococci—in regard to their ability to split sugars is well known, some varieties altering very considerably under cultivation in this respect. This, in fact, was one of the main objections urged against the classification of these organisms by the fermentation of carbohydrates at the time when this method had its greatest vogue.

In the matter of virulence variations are of course notorious, it being generally found that this quality deteriorated during growth upon artificial media. The presence of large quantities of serum in the media was said to delay such loss, and the passage of the organism through the body of an animal to be sometimes effective in restoring it: these observations have no little significance in the light of more recent work upon the serological types of variants. In the case of the diphtheria bacillus differences in virulence are frequent and have in many cases been produced by altering the conditions of growth of the organisms.

The few variations which we have mentioned mostly affect physiological characters, which may be presumed to be more plastic than morphological ones, but variations in the latter are also comparatively commonplace. It is well known that one of the characteristics of the anthrax organism, its branching growth in stab cultures, may be lost after long growth on artificial media: this property of “spiking” is said to be sometimes restored by animal passage, a matter of significance as we shall find when we consider the association of morphological variation with virulence. It is also within the experience of many bacteriologists who have worked extensively at the diagnosis of enteric infections that “inagglutinable typhoid” cultures are some-

times isolated. These may be found either in blood or in stool cultures and, except for their failure to agglutinate, behave in every way like the classical strains. Sometimes, in the same case, an organism from the one source is normally agglutinable whilst one isolated from another source may prove to be inagglutinable. These latter are however, at any rate in certain cases, non-motile, and therefore presumably non-flagellated.

Enough has been said to indicate that bacterial variation is a common occurrence. Usually, as met with spontaneously, this affects only a small proportion of an organism's known properties, so that the practical business of identification is little interfered with; but in some instances, as in the case of inagglutinable strains of dysentery bacilli, the character affected may happen to be an important one and its alteration may give rise to serious practical difficulties.

The study of bacterial variation has to no small extent been retarded by its exponents. So often it has happened that complicated life cycles, including sexual phases, conjugation, ascus formation and the like, have been expounded with such a wealth of detail and a minimum of solid foundation that the sceptic has turned rather impatiently from the perusal of these reports and failed to allow the proper significance which the facts might present if viewed without their more imaginative trimmings.

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Amongst the most serious of the later studies of bacterial variation must be noticed the work of Baerthlein (1918), who produced an enormous mass of detailed results, which had the outstanding merit of clearly demonstrating that colony formation could be correlated with variation in other properties and which served to point the way to much that has been discovered since.

Within the last few years the subject has been attacked again by several groups of workers, prominent amongst whom are Arkwright in this country, de Kruif in the United States, and Weil and Felix in Germany. The results of their efforts have been to lay bare a number of phenomena which were regarded in the first place as being mainly of academic interest, but which are now

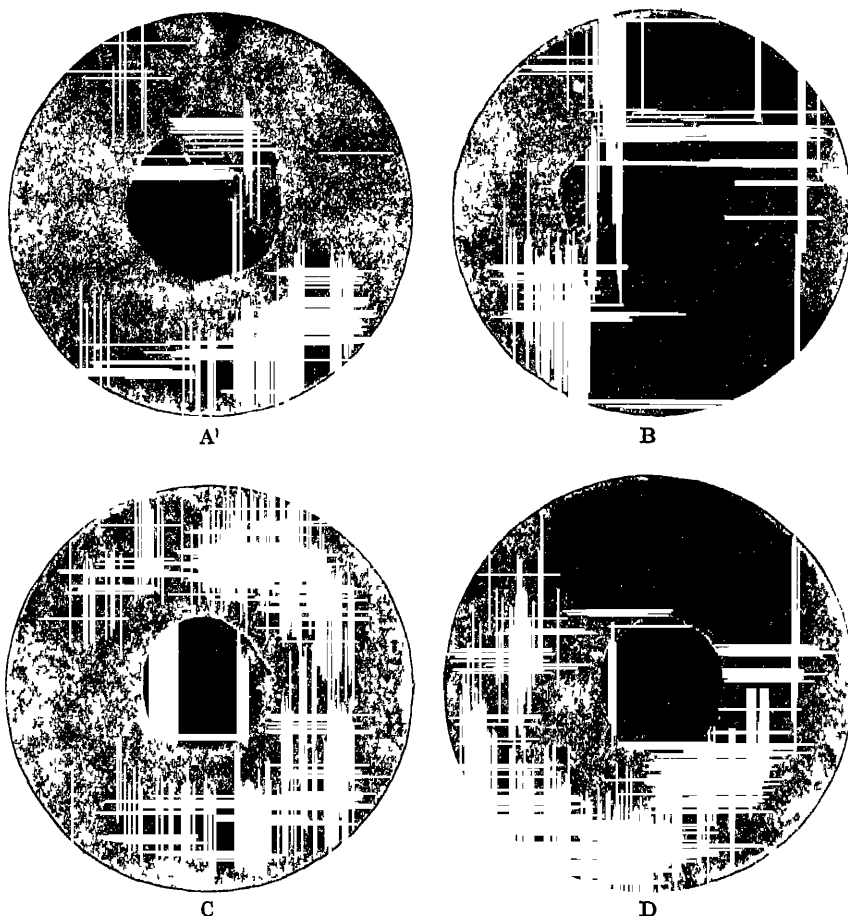


FIG 1—Rough (A and B) and smooth (C and D) colonies of *B. Aertrycke*  
(Topley and Ayrton *Journal of Hygiene*)

appearing to bear very widely upon the practical problems of bacteriology, immunity, and epidemiology.

The work of Arkwright, whose papers on this subject date from 1920, was initially concerned with differences displayed in cultures of bacteria through variations in colony appearance. By plating

old broth, or agar, cultures of laboratory strains of the colityphoid-dysentery group he was able to detect the presence of colonies of two types in what had previously been regarded as a homogeneous culture. Upon agar plates the one type of colony was smooth, round, shining, lenticular and translucent; whilst the colonies of the other form were jagged or irregular in contour, showed a rough matt surface, and were slightly more opaque to transmitted light. These varieties he accordingly designated "smooth" (S) and "rough" (R), from the appearance of the colonies (Fig. 1). The two types were found to occur in *B. dysenteriae*, *Shiga*; *B. typhosus*; *B. paratyphosus* B, and *B. pseudotuberculosis rodentium*; although both types of colony could not be obtained in every instance from each culture examined. Arkwright regarded both of these types as differing from the parent culture, but considered the S form to be more closely related to the "normal" than the R form.

Whilst the strains presenting these morphological differences were similar in their sugar reactions and indol production, an examination of their other properties showed that the change in appearance was associated with profound changes in certain of these. These may be summarised as follows

## SMOOTH TYPE

- (1) Emulsions stable in 0.85 per cent. NaCl
- (2) Growth uniformly turbid in broth.
- (3) Motile.  
(In the case of ciliated bacteria).
- (4) Agglutinated by specific serum in large flocculent clumps.

## ROUGH TYPE

- (1) Agglutinated by this concentration of NaCl
- (2) Growth forms a sediment, with a clear supernatant fluid
- (3) Motility often reduced or absent.
- (4) Agglutinated in small compact granules, falling to the bottom of the tube.

(The two forms described are not always sharply distinct, but would appear to be at the opposite extremes of a series in which

intermediates are not numerous. The sedimented form of growth shown by the R type is due to the salt content of the medium. The agglutination tests are therefore carried out in 0.2 per cent. saline, to obviate the spontaneous agglutination of the R forms in greater concentrations.)

In addition to these properties Arkwright found there were corresponding differences in agglutination tests. Whereas both types agglutinated to titre with ordinary stock laboratory agglutinating serum, yet with monovalent sera prepared from the two types each seemed antigenically specific, at any rate in the higher dilutions. The S forms agglutinated with the S serum and the R forms with the R serum, there being little cross-agglutination. Absorption experiments with the stock agglutinating serum showed it to hold specific and distinct agglutinins, for both S and R varieties. The S strain removed S agglutinins but left those for the R variety, and *vice versa*. These results were generally applicable, but were sharper with some varieties of organisms, e.g., *B. dysenteriae*, *Shiga*, than with others. The S and R types were found to preserve their individuality to a considerable degree when kept regularly subcultured. In some cases changes were observed, the general tendency for these being from the S → R form rather than the reverse. These changes were frequently gradual in nature, the colonies in their intermediate stage showing indeterminate characters.

In later work, examining the pathogenicity and antigenic effects of these two morphological types, Arkwright found that the S forms were pathogenic and the R forms non-pathogenic. Pathogenicity in this case was definitely linked to the colony form and had no relationship to motility, as had been elsewhere suggested. With regard to their immunising properties the smooth forms were the only ones which provoked a satisfactory protective response in inoculated animals, the R forms being of little value in this respect. Arkwright also noted that heating the cultures to 100° C. had little, if any, effect upon their capacity to provoke the immune state. These considerations are intimately bound up with the work of Weil and Felix, and their school, and will be analysed in more detail when dealing with this work (p. 48).

The findings of Arkwright were soon confirmed, in their broad aspects, for certain other species. Mary Cowan demonstrated the same groups for the streptococci and also found the R variants almost completely devoid of pathogenicity. With this group, however, she found that inoculation with R strains was followed by a very considerable degree of immunity against the smooth variety. Griffiths (1928) produced rough variants of pneumococci by growing the organism in its homologous immune serum. Both rough and smooth forms were fairly permanent in culture and the same attenuation in virulence of the rough forms was found, which, however, in this case was associated with a loss of the type-specific soluble substance. Certain serological differences were observed akin to those described by Arkwright, which led Griffiths to the general conclusion that the rough forms were antigenically simpler in constitution than the normal smooth forms. The change to the R form was a reversible one, and the reversion might be brought about through animal passage, the recovery of a smooth morphology being associated with a recovery of virulence. The degree of fixity of the R character, and its associated biological properties, was, however, found to vary in different cases. The evidence obtained pointed to these characters being more firmly stamped on certain colonies than on others, and it was suggested that this depended, to some extent at any rate, upon the circumstances of their production. Griffiths also showed that whilst the S strain was active antigenically, in giving rise to a serum capable of passively protecting mice against the same type of pneumococcus, the R strain was useless in this respect. Reimann (1925) confirmed this work, and extended it to some extent by showing that the R forms of the three recognised pneumococcus types no longer preserved any serological independence, being all agglutinated by a serum prepared from a single R strain. Griffiths, in a later paper (1928), also noted that in the R forms the type distinction could no longer be made out and considered all R forms as essentially the same. Many of these observations of Griffiths', although of course devoid of reference to S and R forms, had been made by Stryker in 1916, who showed that the growth of pneumococci in immune sera resulted in loss of agglutinability, loss of

virulence, loss of capsule formation, and increased phagocytability. It was also noted that mouse passage caused a recovery of the lost properties and that their fixity was determined by the length of treatment with the immune serum

Topley and Ayrton (1924) investigated the matter in *B. enteritidis* (*aertrycke*). They had no difficulty in distinguishing the same rough and smooth types and in showing the very much greater pathogenicity possessed by smooth types than by the rough ones. Bruce White (1925) made similar observations over many members of the salmonella group, and Julianelle obtained the same results with *B. friedlander* (1926). Observations similar to these have been made with an increasing number of species, until the phenomenon is approaching the frequency of a general one. We shall content ourselves with finally noticing the independent work of de Kruif (1921), who worked with an organism of the pasteurella group, *B. leprosepticum*. Approaching the matter from the side of virulence, de Kruif separated off a virulent strain (Type D) and a non-virulent strain (Type G). These, it is evident from his papers, correspond to the S and R strains of Arkwright's terminology, which for the sake of simplicity we shall retain in discussing de Kruif's work. These forms yielded similar differences of growth upon solid media and in broth, the smooth type also proved to be the virulent one, whilst the rough type was non-virulent. De Kruif found however, though his experiments were not extensive, that the avirulent (R) type was an efficient antigen in producing adequate immunity against the virulent (S) type. His agglutination experiments were likewise rather restricted, but led to the general conclusion that the S type was antigenically more active than the R type, although he believed that the difference was only a quantitative one. The two types were remarkably permanent in culture, but working with pure line strains he succeeded in clearly demonstrating that rough varieties were formed in ageing cultures which previously had presented only smooth characters. He also made the important observation that the virulence of a given culture was a function of the proportion of S bacteria present in it and not, as has been widely believed, due to a general raising or lowering of the invasive capacity of each unit in the

culture. In so far as his studies led him, he found that all the S strains with which he dealt were of approximately the same virulence.

It therefore appears, from the work which we have considered, and which is further supported by much that has been left unnoticed, that the existence of S and R forms is well established for a number of species, and that this change in colony appearance is associated with certain other profound changes in the organisms' make-up.

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The work of Weil and Felix, like that of Arkwright, was also preceded by a number of scattered observations at the hands of various workers, pointing to the same conclusions to which their more profound studies have led. Both groups of observations, those of Arkwright and of Weil and Felix, bear mutually upon one another, but for the moment, for the sake of clarity, we shall consider them separately.

Probably the most important of these earlier studies was that of Theodore Smith and Reagh, who, in 1903, investigated the recognised, though then imperfectly understood, influence of flagella upon agglutination. Dealing with a non-motile strain of *B. suispestifer* of which they had become possessed, they found that it agglutinated in a different manner to that in which the ordinary, motile, form agglutinated; showing very small, compact granules, instead of the large, fluffy, floccules of the motile form (their descriptions practically duplicate those of Arkwright). They also found that the non-motile organism required about twenty times as concentrated serum to effect its agglutination as did the motile, and, further, that the non-motile strain was very much less active an antigen for the production of agglutinins in animals than was the motile strain. Up to this point their work closely parallels that of Arkwright and his followers. They found, however, that whereas serum prepared from motile strains was relatively inefficient in agglutinating the non-motile variety, in the converse experiment the serum of the non-motile strain was practically as effective against the motile as against the homologous

organism. The agglutination, however, was of the granular type and not the ordinary, motile, floccular type

Smith and Reagh, therefore, were led to believe that two antigens were present in the bacteria and that two corresponding agglutinins might enter into play. In the case of motile bacteria, agglutinins were provoked, in the injected animals, against the flagellar substance and also against the body substance of the organisms; whereas in the non-motile form, flagella being absent from the antigen, agglutinins were only formed against the corporeal substance of the bacteria. The two observed types of agglutination would therefore depend upon the existence of these different reacting substances; fine, granular, agglutination being produced by the corporeal agglutinins and the commoner coarse floccules by the flagellar agglutinins. This view was to some extent confirmed by the microscopic examination of the agglutinated clumps and by the application of absorption tests, which revealed two agglutinins in the antiserum prepared from motile forms, only one of which was absorbed by the non-motile organisms. One agglutinin, only, was present in the serum produced against the non-motile form, which was capable of absorption by both motile and non-motile organisms.

These workers accordingly formulated the theory of the existence of "flagellar" and body, or "somatic," agglutinogens in the bacteria, and of a corresponding dual nature in the agglutinating bodies produced by their injection.

To turn now to the work of Weil and Felix, which dates from 1917. The starting point of their researches which bear upon the present problem was the observation, made in investigating the agglutination of their *B. proteus* X. 19 by the serum of typhus patients, that this agglutination was not the same in the case of patients' serum as it was in the case of the serum of immunised animals. Whereas the serum of rabbits immunised against bacillus X. 19 agglutinated other strains of *B. proteus*, obtained from several sources, the agglutinating effects of typhus patients' serum was sharply specific for the strain X. 19, and for it alone. A further difference, and one the type of which the reader will by now be abundantly familiar with, was that the typhus serum

agglutinated the organisms into fine uniform masses whilst the immunised rabbits' serum produced larger, coarse, flocculi. Pursuing this observation further, Weil and Felix found that in old cultures of proteus X. 19 were certain colonies which did not show the ordinary, spreading, proteus form of growth, but were compact and discrete. These they designated the "O" type of colony to distinguish them from the ordinary, spreading, "H" type of colony. (H = Hauch; O = ohne Hauch). The O colonies alone were specifically agglutinated by the typhus serum. Serologically they noted other differences between these two types. The O colonies produced a serum giving the fine type of agglutination, whilst the serum made by injecting the H growth gave the coarse type.

By the absorption of sera with these strains they found that the O type removed only agglutinins for itself, whereas the H type removed agglutinins for both varieties, they therefore concluded that two "receptors," or agglutinogens, to use a phrase which does not involve a disputed theory, were present in the H form, but only one in the O. The agglutinating serum produced by the injection of the O type contained a single agglutinin, which gave rise to the fine, granular, reaction and was specific for the O type of organism, whilst the corresponding serum for the H form contained two agglutinins, the small-flaking O agglutinin first mentioned and, in addition, a non-specific large-flaking H agglutinin, active not only against the homologous strain but against other proteus types as well.

These results, whose close similarity to those obtained by Theodore Smith and Reagh will be immediately apparent, were confirmed by others, especially by workers in Weil's laboratory, and were soon extended to *B. typhosus*, the salmonella group, *V. cholerae*, and certain anaerobes. The further investigation of the antigenic structure of these organisms was facilitated by the finding in 1918, by Sachs and Schlossberger, that the H antigen in the bacteria could be destroyed by heating for an hour at from 80–100° C. a procedure which did not affect the O antigen. The general conclusion was accordingly reached by Weil, Felix, and other workers in this field, that the normal *B. proteus* contained

two antigenic fractions; a heat-labile, H, fraction and a heat-stable, O, fraction. The spontaneously occurring O variant contained the heat-stable antigen alone.

Although the variant form, O, which we have been describing was found originally by Weil and Felix to occur spontaneously in laboratory cultures, Felix subsequently obtained this form by growing the organisms at an elevated temperature, and Braun and Schaeffer succeeded in producing it by growing proteus X. 19 upon agar which contained a minimum of nutritive substances, as well as by the use of agar containing 0.17 per cent. of phenol. It was noted that the O forms so produced were eventually non-motile and devoid of flagella. The change from H to O was not abrupt, but a variety of intermediate forms was encountered, both by these workers and also by Felix who has described a multitude of intermediates between H and O, which accordingly would seem to represent the rather well stabilised extremes of a series in which variation is a normal feature.

The heat-labile (H) antigen may be destroyed not only by heating cultures to 100° C., but also by exposing them to certain influences such as weak acids and alkalies, or by treatment with alcohol. The term "stabilotropic" has been suggested by Schiff to designate the O agglutinin, and "labilotropic" for the H agglutinin. It has been shown by Weil and Felix, and their school, that the behaviour of the agglutinins in regard to resistance to heat is the exact opposite to that of the corresponding antigens, the H agglutinin being heat-stable and the O agglutinin labile.

### **THE AGGLUTINATIVE PHASES OF ANDREWES**

Another aspect of the subject, which falls to be considered here, is that elucidated by F. W. Andrewes in the salmonella group. Andrewes, who has uttered a general protest against the common practice of bacteriologists in working with impure re-agents such as ordinary agglutinating sera, which contain besides the specific agglutinins for the antigenic strain of organism group agglutinins of unknown quality and amount, worked with ultra-specific sera obtained by absorbing all save the monospecific agglutinins of

various salmonella types (*B. paratyphosus* B; C; *aertrycke*, and *newport* strains). He made the initial observation that on testing the agglutinability of young broth cultures, made from isolated colonies of the freshly plated organisms, these behaved differently under agglutination. Some showed sharply specific characters, others showed marked co-agglutination.

Further investigation of the matter yielded the information that such cultures fell into two well-defined groups, between which no intermediates were discoverable.

(a) Firstly, the culture under examination might prove to be made up of organisms relatively insensible to group agglutinins, but highly sensitive to specific agglutinins, being agglutinated by mono-specific sera approximately to titre.

(b) In the second instance, the organisms were found to be only weakly agglutinated by specific agglutinins, but readily agglutinated by sera rich in group agglutinins.

These findings are illustrated by Figs. 2 and 3.

The agglutination in all cases was frankly of the floccular type, so that the matter here considered has nothing to do with the two agglutinins of Weil, Felix, and others who have followed them. Andrewes speaks accordingly of "group" and "specific" phases in these organisms, which he

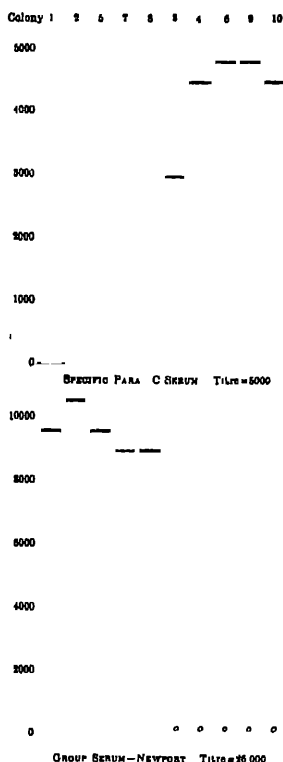


FIG 2—Showing the effect of a specific paratyphosus C serum and a group Newport serum upon various colonies of *B. paratyphosus* C, in the specific phase (8—10), and in the group phase (1—8) —(Andrewes)

generally refers to as "biphasic." It is interesting to note that the organisms appear to be equally pathogenic in either phase.

The colonies from which these differing emulsions came were alike in appearance, and in each case give uniformly turbid suspensions when subcultured into broth, so that there is here no

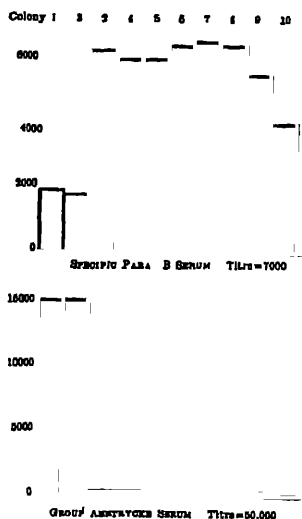


FIG 3—Showing the effect of a specific paratyphosus B serum and a group antityphoid serum upon colonies of *B. paratyphosus B*, in the specific phase (2—10), and in the group phase (1—3) — (Andrewes)

question of rough or smooth forms being concerned. The method of deciding whether in a given colony the organisms are in the specific or in the group phase is simply that of trial, there being no other means of distinction. Individual plates may show colonies in the group and specific phases in about equal numbers, but in general the tendency in Andrewes' work was for the specific phase to predominate. Changes from the one type to the other were found to occur with remarkable readiness, and from no apparent cause, so that subcultivation from the organism in one phase might yield a growth in which it was present in the other.

The general conclusion reached by Andrewes, as a result of these experiments, was that in all mass cultures there exist bacilli of two types: one containing mainly specific antigen, the other mainly group antigens.

There is another side to this work which follows as a corollary

Since it is usually assumed, and with good reason, that the composition of an antiserum is an exact counterpart of the quality of the antigen injected, it should be possible, by utilising pure type-strains, to obtain type sera approximately free of non-specific agglutinins. It is possible to prepare pure specific antigenic strains by testing a number of colonies on a plate, with sera rich in group agglutinins, and subculturing the non-reactors into broth. The young broth emulsions generally retain the specific

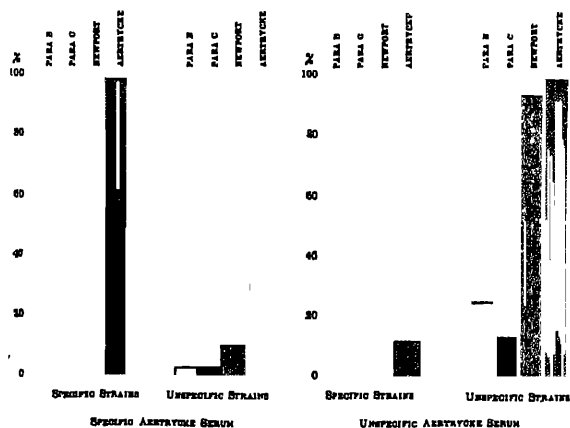


FIG 4—Showing the reaction of the specific and group phases of various salmonellas to specific and unspecific aerrycke sera — (Andrewes)

character, in which case they can be formalinised and either kept in stock or used for injecting into rabbits for the preparation of pure agglutinating sera. The formalinised stock emulsions can also be used for diagnostic purposes, and in Andrewes' hands they have yielded clear-cut results in differentiating between the different paratyphosis B types of salmonella infection, a matter not possible with ordinary laboratory cultures. The sera produced by injecting the type-specific strains are, as should be the case, specific, and have only extremely reduced amounts of group agglutinins. The sharp results which may be obtained, and their differentiating effect upon the group and specific phases of the

organism, are well illustrated by Fig. 4. By completely exhausting the group agglutinins by absorption, pure agglutinating sera may be obtained

In a later paper (1925) Andrewes found the tendency to vary in cultivation from one phase to the other less marked on agar than in broth, and by taking advantage of this he has succeeded in preparing emulsions of the type-specific phase of the organisms in bulk and in carrying out some extremely interesting analyses of their antigenic composition. By using purified type-specific and group sera, and estimating the dosage of bacilli of each phase required to produce a certain drop in antibody content, he has arrived at some quantitative estimate of their antigenic constitution. The fact has been revealed that in the group phase the organism contains only a small portion, about 1 per cent., of specific agglutino-gen. In the specific phase there is some variation between types in the amount of group agglutino-gen which is present, but this in most cases has been found to be exceedingly small.

In the course of his work Andrewes found that the group agglutinogens varied very considerably in the different types of salmonella studied, much more than has been commonly allowed, so that the absorption of serum with one member of the group may have a quite unforeseen effect upon these agglutinins and their complete removal is far from being a certainty. He accordingly postulates, in the antigenic make-up of each organism, certain basic group antigens common to all the members of the group. There are, in addition, further group antigens common only to certain types and, lastly, there are the specific antigens which are peculiar to each type. The quantity of these latter present at any time is a variable matter in the specific phase they entirely dominate the antigenic complex of the bacteria, but on the other hand they are overshadowed and insignificant in amount when the organism is in the group phase

We will leave the further discussion of Andrewes' results, which have received confirmation from Topley and Avton, Savage and Bruce White, and Bensted, until we consider the recent work upon variation as a whole. We would point out, however, that it

raises many practical points of great importance and suggests methods by which purified re-agents may be prepared for the study of closely allied serological types.

It is clear from a study of the work which we have by now briefly outlined, that in many cases the same phenomena have been studied by a number of workers, but by different methods of approach. Arkwright, who used agglutination in salt solution and, secondly, colony form as indications of bacterial variation, succeeded in separating out variants from what, for want of a better term, we may call a "normal" culture. On further investigation he found these morphological types to differ also in such important properties as virulence and antigenic composition. Weil and Felix, basing their investigation upon the last-named property, have separated strains of bacteria which also showed differences in virulence and in colony form, and de Kruif, from the study of virulence, discovered this property to be correlated with the morphological appearance of the colonies of his different strains as well as with differences in antigenic complexity.

There is so much in common in all these observations that the general reader is bound to feel that there is but a single phenomenon in play, which is running through the independent work of these different investigators, and that the S's and R's of Arkwright correspond wholly to the H's and O's of Weil and Felix. There are, however, many difficulties in the acceptance of this simple proposition, and even the proposition itself is far from being as straightforward as it might appear.

In the first place, it should be understood that neither the two main variants described by Arkwright, nor the two in Weil and Felix's work, are isolated types. Although the jump from the one to the other may sometimes be sudden, yet it has frequently been found that both are connected by a series of intermediates; in the experience of some observers by many, in that of others by few. There is consequently the initial problem of when a culture ceases to become S and becomes R, since the qualities of smoothness or roughness are not always lost or assumed at the same time as the correlated properties of virulence and antigenic character

change. There are R's that are at the extreme of variation in this direction and R's that are barely worthy of being called R. The same thing holds, *mutatis mutandis*, for H and O types.

In attempting to correlate the results of different workers it may be stated as evident that both R and O forms are variations in the same direction, since the S and H forms are more or less the starting point from which these others are derived and, if these latter are not actually the "normal" type, they are more closely related to it. Weil and Felix evidently regard their H forms as being the normal, whilst Arkwright looks upon both S and R as variants. By normal we mean the ordinary laboratory mass culture, regarded formerly as a homogeneous population. It is extremely improbable that in this sense any such thing as a normal culture exists, and we should realise that all ordinary laboratory cultures are populations of organisms in which are individuals varying from one another up to the limits of possibility under their conditions of growth. Each culture probably contains its S and R elements, either of which will emerge under suitable conditions, and, as Andrewes has shown, even in cultures apparently homogeneous to the more critical observation, marked variations in antigenic composition may exist between the different constituent organisms.

Given, then, that S and H more closely approximate to our ideas of the classical normal types of bacteria, are they identical? And are R and O, which are the less characteristic forms, likewise identical?

Arkwright and Goyle (1924), in their earlier enquiries, concluded that there was no such identity, since the O antigen which they had prepared absorbed S agglutinins but failed to absorb H agglutinins. They also found that their S type was not appreciably affected as regards its agglutinability by heating. They therefore considered that —

$$\begin{array}{l} S = O \\ \text{and} \quad R = H \end{array}$$

This is a view which might find some support in the fact that they found the S form to be the virulent one, whilst Weil and Felix,

with *B proteus*, found virulence associated with the O form. The view of the identity of S and O, however, was nevertheless contrary to most of the probabilities and opposed to the findings of de Kruijf, Griffiths, Julianelle and others, who all noted that antigenic complexity was associated with the S form, this of course being the main distinguishing feature of Weil and Felix's H.

It would appear that Arkwright and Goyle were misled in believing that their S forms were truly S · since colony form will not serve to distinguish S from O, it is possible that O forms might masquerade under the S guise. Now Arkwright and Goyle grew their S forms upon phenol agar, which is now a known method of producing O variants, and it is therefore probable that in believing they were dealing with S they were actually dealing with O. This is the view which has been put forward by Bruce White to explain the results obtained. He points out that the smooth cultures normally contain H and O antigen, with (in the salmonellas) the H factor predominating, but under certain conditions, as for example culture upon phenol agar, the O factor increases very greatly, and the culture should be regarded and spoken of as O-smooth. White looks upon the change to roughness as involving a marked change in the heat-stable antigen, the culture becoming O-rough, which is quite a distinct entity. In the change to the rough form the H component may be completely lost ; when this happens the loss is permanent, and an independent and characteristic heat-stable antigenic component, R, becomes evident.

Goyle (1926) apparently largely accepts this view, since he recognises two types of smooth colony, a smooth-normal and a smooth-variant, as well as the rough form. He also admits the non-identity of S and O forms, as originally set out by Arkwright and Goyle, and identifies S with H. In his view there are in existence two types of heat-stable antigen, the O type of Weil and Felix, which is species-specific, and an R type, characteristic of the typical rough culture, which is an independent entity and is a non-specific antigen common to a variety of species. This view of the relationship of the differently described antigens is the one also adopted by Arkwright in a recent

paper (1927). The antigenic composition at these different stages in variation would then appear to be much as follows.—

- (S) Smooth normal =  $H + o$
- (S) Smooth variant =  $O \pm r \pm h$
- (R) Rough variant =  $R \pm h \pm o$

(The capital and small letters denote the major and minor antigens respectively )

Arkwright's S is in general the same as Weil and Felix's H, but certain smooth variants belong to the O type, whilst Arkwright's R seems to be a further stage in variation than anything which was reached by Weil and Felix. On his side Arkwright originally overlooked the German workers' O type, being occupied in the main with colony form by which this type is not differentiated. The O form is therefore an intermediate between S and R, allowance being made for certain idiosyncrasies which are found with each type of organism.

The fact that the R antigen is non-specific in character makes it appear probable that these are the antigens responsible for group agglutination. It may well be assumed that Andrewes' group phase in the salmonellas is a condition of the organisms in which the R antigen is well developed, but the character of roughness has not made its appearance in the colonies. This is a reasonable proposition, since it has been abundantly shown that whilst antigenic difference is commonly associated with colony difference, the former may make its appearance without necessarily involving any colony change. The non-specific character of the R antigen is also responsible for the results of Schutze, who found that the R types of various species were all agglutinable to some extent by any R serum, and consequently speaks of the "serological cosmopolitanism" of all rough strains. This tendency of convergence of types along with the assumption of the character of roughness has also been noted in the pneumococci (Griffiths, Reimann). The "O" heat-stable antigen is unlike the "R" in that it is specific for the species in question in each case.

**Source of "H" and "O" Antigens.**—The evidence for the

existence of these reactors has been summarised and we may now consider some observations bearing upon their source. For this we are largely indebted to a paper by Arkwright (1927). The normal, motile, forms of the salmonellas contain both heat-stable and heat-labile agglutinogens, and on injection into animals give rise to sera in which corresponding agglutinins are similarly present. If however such a serum be prepared against one of the non-motile variants of these organisms, it is found to contain only the (stabilotropic) type of agglutinin which occasions the characteristic, finely granular, form of agglutination. The experiment has been carried out of vigorously shaking up and centrifuging emulsions of the motile bacteria, so as to obtain a fluid rich in the torn-off flagella but devoid of the bacterial bodies. Such a preparation agglutinates with serum in the floccular fashion characteristic of the H agglutinin and is, moreover, heat-labile. It therefore appears probable that the "H" agglutinin is bound up with the flagella of the bacteria, and by means such as this can be obtained free from the heat-stable form, which is a somatic antigen contained in the body-substance of the bacteria. This view, it will be seen, is in reality that of Theobald Smith and Reagh (p 48), brought into line with more recent work. It seems probable that it is chiefly the O antigen which has been the heat-stable one dealt with in most of the work upon somatic and flagellar agglutinins. The R antigen is evidently of the somatic type also and agglutinates in similar granular fashion.

The matter is well summarised in the following table, borrowed from Arkwright and slightly modified :—

Antigens or factors	Heat-labile or stable at 100° C	Flagellar or Somatic	Serum Agglutination	Salt Aggluti- nation	Present in Variants
H	Labile	Flagellar	Large flocculi	—	S M. & R M
†S (=O)	Stable	Somatic	Small, granular	—	S M & S N M
R	(Stable)*	Somatic	Very small, muddy	+	R M & R N M.

\* The R is less heat-stable than the O in some species.

S M = smooth motile.

R M. = rough motile.

S N M = smooth non-motile.

R N M = rough non-motile.

† S = Smooth variant

The very obvious differences in the types of agglutination and the part played by the flagella can readily be observed under the microscope. Under the action of the H agglutinins the bacilli gradually collect into loose clumps, with a narrow clear space between the individual organisms. They rapidly become immobile when once entangled in a clump. In the somatic type of agglutination the bacillary bodies are densely packed together, usually in small clumps free action of the flagella continues, and the smaller clumps may be seen actually swimming about

The assumption that the heat-stable and heat-labile antigens are identical with the flagellar and body substance of bacteria is not universally applicable, since non-flagellated organisms can similarly be shown to be possessed of dual antigenic character, e.g., *pneumococci*; *B. friedlander*. In the case of the last-named organism the work of Julianelle (1926) showed the double antigen to be present in the normal forms of the organism but absent from the R variants, which had also lost their capsules and type specificity. It was therefore suggested that the specific antigenic substance resided in the capsule. But even this modified view will not cover every case, since in uncapsulated organisms the dual antigen may be present, as has been found in *B. dysenteriae*, and, as long ago as 1910, was noted by Bordet and Sleeswijk in *B. pertussis*.

### THE ASSOCIATION OF VIRULENCE WITH MORPHOLOGICAL VARIATION

We have already mentioned de Kruijff's observations on the virulence of the smooth strains of *B. leprosepticum* and their bearing upon the general problem (p. 42). In the salmonella group virulence has almost constantly been an attribute of the smooth strains, the roughs being very deficient in this quality. Aik-wright has found that it is the quality of smoothness which is definitely linked with virulence, the question of motility not entering into the matter at all, and, since heat-labile (H) antigen is present in both virulent and avirulent forms, whilst the O antigen alone is common to all virulent forms, he assumes that

this (O) factor has an all-important bearing upon virulence. It may be recalled here that Weil and Felix found that the O form of proteus X. 19 was much more virulent than was the H form.

The work of Griffiths upon the pneumococci has also firmly established the strikingly virulent character of the smooth types and the no less strikingly avirulent character of the roughs, which failed to kill mice in doses of as much as 0.25 c.c. of a blood broth culture, whilst the corresponding smooth type might prove fatal in doses of  $10^{-7}$ – $10^{-8}$  of a cubic centimetre. In cases in which the R type, in large doses, proved fatal the organisms recovered from the blood were usually of the S type, reversion having taken place. Griffiths also made the important observation that the change from S to R in the pneumococci was associated with loss of specific soluble substance for the type in question, and it is possible that it is this which is the essential factor in determining virulence. Julianelle, working with *B. friedlander*, adopted the lines previously followed by Griffiths and obtained very similar results. He produced avirulent R types by growing the organism in immune serum, and in this species also he was able to demonstrate a specific soluble substance akin to that found with the pneumococci. The R types of organism were non-capsulated, avirulent, and did not produce this substance, whilst sera prepared from them failed to precipitate with it. The R strains, in losing their specificity and virulence, seemed to acquire common properties with other types of coliform organisms.

It appears possible, however, that there may be exceptions to this general rule and that in certain groups of organisms the association of smoothness with virulence may not hold, and that individual exceptions also may occur. Cowan described roughness in streptococci as being associated with lack of virulence, which is a conclusion generally contrary to clinical experience, if roughness is contingent upon chain formation. On the other hand Andrewes, in a communication to the Pathological Society of Great Britain and Ireland, in 1928, definitely associated the property of roughness in this group with virulence, and Todd (1928), in the investigation of a single strain of streptococcus,

found that a virulent hæmolytic type yielded after prolonged subculture both rough and smooth varieties. The latter were permanently avirulent, whilst the former, whilst avirulent when first observed, were susceptible to acquiring this property as a result of passage.

It is not impossible, however, that what is described as "roughness" by these differing observers may not always be the same property. Griffiths (1928) noted a rough strain of pneumococcus which was both virulent and a producer of specific substance. In the case of the anthrax bacilli a rough form of colony (*caput medusæ*) is usually found in virulent strains, whilst various of the avirulent mutants which have been described are definitely smooth and domed in their colony characters. Two possibilities present themselves. In the sorting out of characters which undoubtedly takes place in bacteria in different phases of variation, and which results in certain now well-known associations, exceptions may occur, and a character which normally goes with one type of colony may become divorced from this and associated with its opposite. A second possibility is that in organisms such as the streptococci and anthrax bacteria, in which the constituent units in the colony are often relatively very large, a rough appearance may be based upon this fortuitous circumstance and have nothing in common with the little known changes which are responsible for the colonial appearance of roughness amongst the smaller non-agglomerate bacteria.

**Antigenic Constitution and Immunity.**—The discoveries which have been made in this connection are amongst the more practical outcomes of the study of variation. It has been a general experience that agglutinins are produced with greater difficulty, and that the sera generally have a lower titre, as a result of the injection of R forms than of H or O types. Griffiths (1928) investigated the protective action of S and R pneumococcal antisera upon mice. He found that the serum produced by the S variety, when injected intraperitoneally into the mice in 0.2 c.c. doses, was very effective in protecting them against subsequent injections of highly virulent cocci of the same type (Types I. and II.), protection being given against 0.1 of a cubic centimetre of a broth culture capable of

killing unprotected animals in a dose of  $10^{-8}$  c.c. All sera produced by R strains were completely ineffective.

Arkwright (1927) has very thoroughly investigated the value of the different antigenic types as enteric vaccines, in the case of *B. paratyphosus A*, and has obtained results essentially in agreement with these. The tests were carried out upon guinea-pigs which were inoculated with various of the recognised antigenic types and were tested for resistance two to three weeks later by means of an intraperitoneal injection of from three to five fatal doses of a virulent broth culture of the organism. The results may be summarised as follows from Arkwright's figures :—

Experiment.	Vaccine Constitution.	Number of G Pigs tested	Number which survived Test Injection.	Per cent surviving
I.	H + O	13	11	85
II.	O	38	33	87
III.	H + R	22	10	45
IV.	R	8	0	0
	Controls	20	2	10

(Arkwright uses the term S in place of O which appears in this table. The two are probably in this case interchangeable expressions, but he prefers the expression S to avoid confusion, since the R antigen, also heat-stable, was not recognised by Weil and Felix. The term O has been employed here in following out previous usage.)

It will be seen from these results that the best protection was afforded by the vaccine containing the O element ("S" Arkwright), and that the absence of the H element in the second experiment shown above has not affected the immunising power of the antigen. It was also found that the protective power of the O-containing antigen was in no way lowered by heating to  $100^{\circ}$  C. for half an hour, so that the heat resistance of the antigenic substance was comparable to that of the O substance, and therefore good reason was forthcoming for assuming the close relationship if not the identity of these. Similar experiments

with *B. typhosus* gave results of the same order, but not quite so striking, this probably being due to the greater virulence of the typhoid bacillus for the experimental animal.

The practical outcome of these findings gives much food for thought. Where attention has been given to the constitution of anti-typhoid vaccines it has by no means always been considered either desirable or necessary to employ organisms whose virulence is high and, as Arkwright points out, the British Army Council Anti-typhoid Committee did not consider virulence an essential in the organisms used to prepare typhoid vaccine. It is now apparent, however, that it is the O antigen, the characteristic stamp of a virulent strain, which alone has well-developed powers of provoking protective immunity, and that strains in the rough state may be quite inert. Possibly the prejudice in favour of autogenous vaccines may herein find its foundation, the better results attributed to those being less a virtue of any special strain than due to the fact that the O element is more likely to predominate in a newly isolated culture than in old laboratory stocks, which may have moved considerably over to the R side.

A second important point, which has been clearly demonstrated by Arkwright, is the negligibility of the effect of heat upon the antigenic value of vaccines. The O antigen is sufficiently stable to withstand heating to 100° C. for at least half an hour, and the protection afforded by vaccine so prepared was every bit as potent as that given by any of the vaccines exposed to lower temperatures.

These results run absolutely counter to many venerable bacteriological superstitions, in respect to which vaccines have been killed in many tender ways in the hope of preserving a maximum of antigenic value. Though their future extension may greatly modify bacteriological practice, it should be remembered that at the moment they have been obtained with only a very limited number of the pathogenic bacteria and, as Arkwright himself insists, it would be foolish to be blinded by the newly recognised importance of the O element to the possible rôle played by other and unknown substances, which may be affected by high temperatures.

Rather a different aspect of the subject has been developed by

Felix (1924-27) in connection with typhoidal disease. He maintains that the clinically important agglutinins in this condition are the small-flaking ones which, as we have previously seen, are related to the heat-stable agglutinogens. In the case of ordinary anti-typhoid or T.A.B., vaccination, he states that the agglutinins developed are of the large-flaking variety because of the practical absence of O agglutinogens in the vaccine. (It is hard to conceive of their complete absence.) In the disease itself, on the other hand, O agglutinins appear in the patient's serum, and by their separate titration he believes that the serological diagnosis can be made with a single test in an inoculated subject. The small-flaking agglutinins are held by Felix to be the true protective antibodies and to pursue in the blood an inverse course to the bacteræmia. Where they are absent, or only present in very small amounts, the bacteræmia is marked, but with their appearance in considerable concentration the bacilli disappear from the blood. The bactericidal power of the blood is believed by Felix to be related to the presence of these agglutinins, and he maintains that the widely held opinion that the agglutinin content of the blood gives no accurate index of immunity is erroneous and due to attention having been focussed mainly upon the more easily observed large-flaking agglutinins. It may be also mentioned here that a number of workers have found the O antigen and the corresponding stabilotropic antiserum to be the factors mainly concerned in complement fixation, and the opsonic phenomena (Braun and Nodake, 1924 ; Felix and Robertson, 1928.)

Reviewing the whole matter in as broad an aspect as possible, it is clearly evident that modern work has disclosed an unsuspected degree of variation in bacteria which appears to be in the main latent, though upon this point further knowledge is desirable, but is capable of being brought into evidence as a result of certain alterations in the environmental conditions. Ageing cultures, starvation by growing the organisms on media poor in nutrient substances, the action of chemicals, or of dissociating agents such as the bacteriophage, and of anti-substances such as immune sera,

all permit the appearance of forms differing in important respects from the "normal" type. It is also the case, though a matter which we have omitted to consider from limitation of space, that other properties are involved in this variation besides those which have been enumerated. Chromogenesis, the morphology of the individual bacilli, capsule formation and sporulation, are all properties which may be found modified in the variant types. It seems that in general these properties, as well as those which we have previously considered, tend to be lost in the variant types which have been most widely studied. The properties of roughness and smoothness, which are perhaps the most convenient indicators of variation, seem to be moderately stable phases in a definite variational trend. With the acquisition of roughness, which is the simplest and possibly the best of our present criteria of variation, and indicates that a marked change has taken place, it is found that mobility tends to disappear, virulence goes, antigenic capacity is lost, serological independence is blurred, and a rather simpler and more vegetative phase in existence is arrived at. There seems therefore to be a definite tendency towards negativism in bacterial variation as it has been observed in most of the work which we have reviewed. This, of course, may be due to the somewhat stereotyped conditions to which the organisms have been exposed under laboratory conditions, and it is eminently possible that in very different circumstances variation in an altogether different direction might result.

It is a speculation of extraordinary interest to consider the possibility of variation in the opposite direction, the factors which condition this, and what its results might be. The experiments of Griffiths showed that this could take place, since he found that at times avirulent, rough, strains of pneumococci might kill mice and in the process the corresponding smooth form emerge. The common directional trend of variation had been reversed, and from the rough avirulent strain, with its usual contingent properties, the smooth virulent strain had been obtained, with all the coincident implications of smoothness. Similar results have been got by others, so that we know the  $R \rightarrow S$  transformation to be freely possible, although more difficult to obtain in the laboratory than

the converse is. The matter is of much importance in relationship to disease and epidemiology. It is so commonplace, as to be almost a law in bacteriology, that wherever pathogenic organisms are found, there their kindred brethren of a non-pathogenic type are also present. Although many of these latter have the fixity of species, it is by no means uncommon to find others which are indistinguishable from the real pathogenic varieties save by lack of virulence or the fact that they do not invade. Should these prove to be non-virulent mutants of the pathogenic type, a very interesting field of work is opened up in the investigation of the causes of disease and the rise of epidemics. On the other hand, on the understanding of the decline of an infection the newer studies of bacterial variation may well have an important bearing.

We have long been very content to point to the evidence of antibody production, but our knowledge of the interplay between this and the organisms is woefully scanty. Here again light may come from the effects observed by Griffiths and others upon the variations produced in bacteria by growth in immune serum. The result, as previously mentioned, was the formation of the rough avirulent types, a finding which has received confirmation in the case of Friedlander's bacillus by Julianelle and in *B. subtilis* by Soule. These results are not by any means in accord with what has always been taught or found, although it may be recalled that Metchnikoff showed that anthrax bacilli became deprived of their virulence for rabbits by growth in the blood of immunised sheep. The contradictory results obtained by others in this connection are possibly due to widely differing conditions of experiment, such as strength of the immune serum, bulk of the culture, length of contact, or other of the many variable factors involved. It may also be objected that such a view, which contemplates the attenuation of organisms by conditions affecting them *in vivo*, is contrary to the great mass of evidence adduced from passage experiments, which points to growth in the animal body having the effect of exalting their virulence. This is not necessarily valid, however, for in passage experiments the point usually investigated has been the degree of virulence of the organism in animals which have died; *i.e.* they merely show the results of the growth of

bacteria in an animal in which the defence has failed and which has proved a very suitable environment for their development and provided an abundant pabulum. The question of what might be their condition in animals which have failed to die has been neglected. The results of a negative experiment are rarely as attractive as those of a positive one!

Many a person may have asked himself how an epidemic infection ever dies out. On the basis of a mass of laboratory evidence the organisms, by a process of continued passage, should go from strength to strength until they finally achieve a degree of virulence certainly fatal, and the process of elimination of susceptible individuals, often invoked, can rarely go on to the extent necessary for it alone to terminate the infection. If the view may be adopted that under the influence of antibodies in resistant cases the organisms undergo variation in the R direction, then recovery from the disease and the course of epidemic infections seems singularly easier to understand. The view is no new one, having been formulated as long ago as 1892 by Roger, who suggested that under the influence of antibodies bacteria were not killed off but became avirulent.

It may therefore be said, in conclusion, that we have arrived at a stage in our knowledge at which it seems evident that, in order to infect, the organism must be in a certain condition or phase which involves possession of the O antigen. We also know that such a condition is the one in which infection will provoke a maximum of immunity response and, further, that in certain cases it has been clearly shown that under exposure to antibodies, generated in this way, the bacteria may enter another phase, in which their predominating antigenic constituent is the R factor and in which they are no longer virulent.

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## CHAPTER IV

### THE BACTERIOPHAGE

Most discoveries have their roots deep in the past. In 1896 Hankin published a paper in the *Annales de l'Institut Pasteur* in which he drew attention to the notable bactericidal properties of certain Indian river waters. He found that the Jumna, just below Agra, contained over 100,000 organisms per cubic centimetre, but that five kilometres further down the river only contained 90–100 organisms in the same bulk. On further investigation it was found that the filtered water exerted a marked lethal action upon the cholera vibrio in the test tube, an effect which was abolished by boiling. Hankin attributed this to a volatile substance, which he failed to isolate, and his observations remained lonely and unexplained until brought into light again by d'Herelle.

In 1917 d'Herelle made the fundamental observation which led to the discovery of the bacteriophage, about the phenomena concerning which an enormous mass of literature is now assembled. Working at the Institut Pasteur, d'Herelle discovered, in sterile filtrates from the stools of a patient suffering from Shiga dysentery, a substance which inhibited the development of these organisms in broth cultures and, under certain conditions, destroyed them. If, for example, a few drops of such a faecal filtrate, active in this manner, were introduced into a young broth culture of *B. dysenteriae Shiga*, in which the clouding of bacillary growth was just beginning to appear, and the mixture were further incubated, the result was not the usual and progressive increase in the turbidity of the culture but a gradual clearing, and after twelve to twenty-four hours all trace of bacillary growth had disappeared. The bacilli were no longer detectable and, according to d'Herelle's early statements, were completely killed off.

The striking and unique feature about this phenomenon, and

one outside the realm of previous experience, was that the lysis was found to be transmissible in series, *ad infinitum*, without any exhaustion of the lytic principle. At the end of such an experiment the principle could be separated off by filtration, and the resulting sterile filtrate used to start the lysis of a second bacterial culture. This was an entirely new phenomenon in bacteriology, quite different from any form of bacterial destruction hitherto observed, and d'Herelle quickly proclaimed it to be brought about by an ultra-microscopic living virus, a parasite of the bacteria, which underwent multiplication in the cultures whose dissolution it caused. In his view these solutions were in effect cultures of the virus, to which he gave the name of *Bacteriophagum Intestinale*. Such a position he has maintained unmodified, in the face of a great deal of opposition, up to the present time.

A second method of showing bacteriophagic activity, and a very important one for the detailed study of the phenomenon, is that devised by d'Herelle for its demonstration in surface cultures. D'Herelle found that if a very small quantity of a lytic filtrate, or of a lysed culture such as we have just described, were added to a young broth culture of the Shiga bacillus and subcultures upon agar made from the mixture at regular intervals, then at a certain period of their interaction the result obtained was a surface growth of the bacillus on the agar which showed sharply punched-out clear areas which were devoid of organisms (Fig 5). These areas d'Herelle claimed to be "colonies" of the bacteriophage, and from this experiment he devised a technique by which he considers it possible to estimate

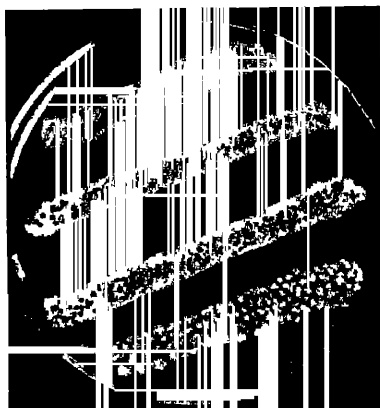


FIG 5 —Areas of bacteriolysis in surface growths of *B. coli* —(After Caldwell)

the number of bacteriophage units in any given fluid. The method is also available for the demonstration of a feeble bacteriophagic action, which would be too slight to be detected by the visible lysis of a broth culture.

Such, in the barest outline, are the fundamental experiments upon which all the work and theories which the subject has called forth are founded. These are accepted facts and outside dispute.

We have purposely, for the moment, omitted any mention of the work of F. W. Twort, whose name is so frequently conjoined with that of d'Herelle as co-discoverer of the phenomenon. Twort, working upon filtrable viruses and especially with vaccinia, discovered in 1915 that certain colonies of cocci which he had cultivated from vaccine lymph underwent a glassy degeneration, becoming transformed into a transparent vitreous substance which, on staining, showed only unrecognisable débris and no proper coccal forms. He further found that this degeneration was a contagious condition in the colonies, and could be transmitted from "diseased" to "healthy" colonies by inoculation with the platinum wire. The infective material was capable of passing Pasteur-Chamberland B filters and of transmission through an infinite number of generations. Looked at in a broad way, these results appear practically identical with those of d'Herelle, although obtained under different circumstances and with a different type of organism. Twort himself appears to consider the phenomena identical, as also does Bordet, but this is strongly contested by d'Herelle mainly on the grounds that in his experiments the destruction of the bacilli is absolute, and no residue is left, whilst Twort speaks of a transformation of the colony into a glassy substance. Further, the bare plaques produced on agar by the interaction of his bacteriophage and the bacilli are static and show no increase in size, whilst in Twort's experiments a progressive invasion of the colonies of cocci is described. To the outside observer of a rather acutely waged dispute it appears that there are certain differences in the two phenomena, although both are alike in that their describers have both discovered a transmissible form of bacterial lysis hitherto unrecognised.

**The General Characters of the Bacteriophage.**—These are varied and extraordinary. In the first place the principle is non-specific, and its action may be exerted upon a number of bacterial species, usually, however, not to the same extent upon each. Thus a given bacteriophage may have a powerful dissolving action upon Shiga's bacillus, a moderate one on *B. paratyphosus* B, and a slight action upon *B. coli*, whilst the intensity with which another strain affects these organisms may be in the converse direction. In the second place, the ability of any strain of bacteriophage to lyse microorganisms is susceptible of being altered; its virulence may be enhanced, in d'Herelle's phraseology. This is done by repeated transfers in broth culture, during which any increase in activity of the bacteriophage can be gauged by the number of the bare plaques formed in cultures made upon agar.

Bacteriophagic activity was first observed against dysentery bacilli, and it is mainly in connection with the colon-typhoid-dysentery group that the phenomena have been studied and active strains most readily obtained. Nevertheless the extent of the phenomenon is by no means limited to this group, but has been found to embrace the whole range of intestinal organisms, the salmonella and pasteurella groups, *V. cholerae*, *B. subtilis*, *staphylococci*, *streptococci*, etc.; although the Gram-positive organisms are in a general way less susceptible to the lytic agent than are others.

The bacteriophagic substance becomes increased in the course of its action. This d'Herelle deduces from a comparison of the number of bare areas present in plates spread from mixtures of bacteriophage and organisms during the course of their interaction. If tubes of a suspension of Shiga bacilli be inoculated with very minute quantities of the bacteriophage, and regular platings carried out during the subsequent incubation, in the hours that follow the number of bare areas on the resulting plates will become progressively more numerous, coincident with the clearing of the broth cultures, until finally the plates fail to show any growth of bacilli. The bare areas, as we have already indicated, are regarded by d'Herelle as "colonies" of the bacteriophage. This contention he supports by the fact that the areas contain the lytic principle, and inoculation from them into young broth cultures of suscep-

tible organisms leads to the lysis of the latter. The clear fluid which results from lysis is therefore regarded by d'Herelle as a pure culture of the bacteriophage. For the production of the phenomenon living cultures are necessary. No bacteriophagic action can be demonstrated on dead bacilli. Twort (1925) showed that dead staphylococci, exposed to the lytic agent, underwent lysis if living cocci were present along with them. This experiment has been further investigated by Bronfenbrenner and Muckenfuss (1927), who have found that the conditions in which this takes place are very special, and that if the living and the dead are kept apart by the interposition of a semi-permeable membrane no dissolution of the dead bacteria will occur, although the bacteriophage diffuses freely through the membrane. The lysis therefore of the dead bacteria is not directly attributable to the action of the bacteriophage but, according to these authors, to a ferment set free from the disintegrating live organisms. They were able to detect a substance of this nature, producing non-transmissible lysis of dead bacteria, in cultures which were undergoing spontaneous autolysis. This possibly is the same as the "lysozyme" described by Fleming (1922) in a variety of organic products.

**Isolation and Distribution of the Bacteriophage.**—The bacteriophage is ubiquitous in its distribution, but is always found in association with living bacteria. It has been isolated from the faeces of normal persons and animals, as well as from those of persons convalescent from intestinal disorders. It has been found in water and in soil, in sewage, in urine, in pus, in trypsin, in peptone, etc. Bordet obtained it from the leucocytic exudate in the peritoneal cavity of an animal injected with bacteria. The isolation of the principle may be effected by filtering the homogenised materials through bacterial filters or by repeated heatings to 56–58° C., which destroy non-sporing organisms but not the bacteriophage. Its action, however, is weakened by this process, so that the filtration method is the one to be preferred. Hadley (1928) states that it may in certain cases be obtained by the simple procedure of growing an organism repeatedly in its own filtrates, over fourteen to twenty generations.

**Physical and Other Properties of the Bacteriophage.**—The action of the lytic agent is only exerted on young, actively growing, cultures. This takes place over a fairly wide range of H. ion concentration, but is most active just on the alkaline side of neutrality. Gratia gives 8.5 as the optimum pH for lysis. Acidity of the medium generally has a deterrent effect upon the process. The bacteriophagic principle is filtrable with ease through the whole range of Pasteur-Chamberland filters. It will also pass the coarser colloidal filters, which will allow the passage of collargol particles (Praustnitz, 1922). The particulate nature of the agent, or its association with particulate matter, therefore seems fairly certain, and according to d'Herelle a filtered suspension of it gives the Tyndall phenomenon. Concentration can also be effected by centrifugalization at high speeds, though this point is disputed. The effects of noxious agents, and of keeping, upon a bacteriophage preparation, according to d'Herelle, are difficult to follow accurately, since inactivity of the preparation may be due either to "death" of the corpuscles or to "loss of virulence," the latter being recognised as an unstable quality. In general it may be said that the activity of the substance persists over a number of years. The difficulty alluded to above is also encountered in estimating the destructive action of physical agents and antiseptics. In many such experiments a possible action of the substances under investigation upon the bacteria themselves has not been fully taken into account. The lytic material is, however, rendered completely and permanently inert by exposure to a temperature of 75° C. for thirty minutes. The activity of the substance is rapidly destroyed by ultra-violet light, but towards chemical agents it shows considerable resistance, this, according to d'Herelle, being intermediate between that of the vegetative forms of bacteria and the spores of *B. subtilis*.

#### THE EFFECT OF THE BACTERIOPHAGE UPON BACTERIA

The first result of the contact between bacteriophage and bacterium is the adsorption or absorption of the bacteriophage by the bacterium. This has been shown by Lepper (1923), who was

able to demonstrate this combination at low temperatures without lysis ; this, however, rapidly took place when the temperature was raised.

Viewed under the dark background the involved bacteria, *e g.*, *B. dysenteriae* (*Shiga*), are seen to swell up and become spherical. Suddenly they burst and disappear in a cloud of fine granules, which gradually disperse. Formerly d'Herelle regarded these granules as the bacteriophage in visible form, but more recently he has abandoned this view. In the case of marked bacteriophage activity the whole of the bacteria in a suspension may be destroyed, but it more commonly happens that, although destruction appears complete, some of the organisms survive, and if incubation be continued growth begins to reappear again after an interval of a few days. The frequency with which this occurs is inversely related to the activity of the strain of bacteriophage employed. Subcultures of such surviving organisms give growths of the original bacterium upon which the experiment was carried out, but these often show marked differences from the typical parent strain. Such growths are termed by d'Herelle "secondary cultures."

The course of the destruction of bacteria by the bacteriophage does not always proceed uniformly forward, but varies in accordance with the conditions of the experiment. In some cases it was found by Gratia, who worked at the lysis of bacteria in rather acid media in which there is a general slowing down of the phenomenon, that it proceeds in a series of waves of growth and resolution, each successive wave of growth being more marked until, under the conditions of his experiment, the final result was the establishment of a secondary culture which was wholly resistant to bacteriophagy. According to Gratia the ultimate survival of some members of the bacterial population is a matter of selection, and not of adaptation as believed by d'Herelle. The organisms which develop in a secondary culture show considerable differences from the parent strain. In cultures made upon agar two chief types of colony may be found. Although all the survivors do not fall into these classes, the predominant types are :

(1) Strains merely resistant to lysis.

(2) Of greater interest are certain strains which, whilst themselves resistant, have incorporated with them the lytic principle, which remains present through future subcultures. These are the "lysogenic" strains of Bordet and are described as "contaminated" colonies by d'Herelle. Such cultures are capable of initiating lysis in growths of ordinary lysable bacteria, by the inoculation of the latter with a trace of the lysogenic strain. The susceptible organisms undergo solution; the lysogenic strain persists and ultimately grows in the medium.

The secondary cultures often tend to grow ill in fluid media and to form agglutinated masses at the bottom of the tube. On agar the growth is scanty, the colonies discrete and of a sticky mucous consistency. These strains are not agglutinated, or are only partially agglutinated, by normal agglutinating sera for the type organism (d'Herelle). Bacilli tend to take on coccal shape and may lose their motility. Their virulence for laboratory animals is generally greater (d'Herelle, Border, Gratia, etc.), although there is some disagreement upon this point, as there well may be in view of the number of different types of secondary cultures which can emerge as a result of bacteriophagic action. Gratia, indeed, has shown that the characters of an organism may be so variously altered that as many as eleven different forms may arise out of a single culture from the action of the lytic agent.

The lysogenic character of the secondary cultures which show it is not permanent, and by repeated cultivations from isolated colonies d'Herelle states that the "original" type is again produced by the elimination of the bacteriophage. Bordet and Ciuca report some rather striking experiments in which they submitted such altered bacteria to an antiserum against the bacteriophage prepared from rabbits, which had an inhibitory action upon the agent *in vitro*. The effect of this was to restore immediately the original type of colony, in so far as morphology was concerned, and to destroy its lysogenic power. The organisms, however, remained resistant to bacteriophagy, and only became sensitive again after a series of subcultures. Bruynoghe (1928) obtained somewhat similar results, which,

however, pointed to the effect of anti-bacteriophage serum being upon the bacteria rather than on the bacteriophage.

It is a matter of great interest to know what is the relationship between these "secondary cultures" of d'Herelle and the variants described in recent years by others (Arkwright, Weil and Felix, etc.), as occurring under different conditions in bacterial cultures (see Chapter III).

Certain changes, such as loss of motility, or of agglutinability, and the acquisition of resistance to lysis, might be interpreted as indicating a trend towards the rough type. This form of change, however, which Hadley (1928) sees to be the essential one in the evolution of the resistant colony, is not necessarily complete, and in the experience of many workers increased virulence has been a character of such resistant colonies with which they have worked. This is not at all in keeping with R characters, although it is with the O ones. Arkwright (1924) obtained some curious results, which do not simplify the matter. Examining six strains of Shiga's bacillus, none of which originally underwent lysis by the bacteriophage, he separated off smooth and rough types. From the descriptions of the latter they would appear not unlike d'Herelle's resistant strains. These were all free from bacteriophage contamination, but they were the only ones which were sensitive to lysis; only certain of these rough variants were acted upon. It has since been shown by a number of workers that in general the rough strains are more resistant to bacteriolysis than are the smooth. The importance of Arkwright's work lies rather in the fact that he showed clearly that variants could be obtained, which differed in their sensitiveness to the lytic agent, by methods of selection other than its action.

From the large number of variants which can be isolated upon agar as a result of the action of the bacteriophage, Arkwright believes that we are not dealing with the selection of those previously existing; in this he is in agreement with d'Herelle, though fundamentally differing from him in his view as to the nature of the bacteriophagic process. He regards the bacteriophage as a stimulant to variation, especially in certain directions, a conclusion which is eminently reasonable since it has been

established that the active period of the bacteriophage is that in which the bacteria are dividing. In Arkwright's view tendencies to variation, which are latent under ordinary cultural conditions, profit by the removal of inhibiting factors, or the presence of exciting ones, and take effect.

As an illustration of the complexity of the subject, it may be mentioned that Bronfenbrenner, Muckenfuss and Korb found that the resistant strains which they obtained of *B. pestis caviae* were avirulent, whereas the sensitive form were pathogenic to a high degree—a result in direct contradiction to those of d'Herelle and a number of others. The workers here cited were also of the opinion that the effect produced was one of selection amongst variants already pre-existing in a culture.

Gratia (1928) has brought forward evidence that different types of bacteriophage may exist and that the nature of the secondary colonies is determined by these. He bases this belief upon the finding of strains of bacteriophage which yield either large or small plaques upon agar. He finds that the secondary colonies which appear after the action of one of these types are sensitive to the lytic action of the other, and *vice versa*. The two types of resistant colony, which correspond to the two types of bacteriophage may be of the S and R varieties. Where both forms of bacteriophage act together the lytic action is more marked than where one alone is in action. Gratia further found that antiserum produced against a large-plate bacteriophage would neutralise this completely, but would only partially neutralise the small-plate type, and *vice versa*. According to Hadley, who denotes the bacteriophages which produce large and small plaques as  $\alpha$  and  $\beta$  respectively, the lytic action of the  $\alpha$  type is limited to the S variety of bacterial colony, and to certain types of secondary cultures which survive  $\beta$  bacteriophagy. The  $\beta$  bacteriophage for its part is lytic for S cultures and for certain secondary colonies resistant to  $\alpha$ . The survivors of  $\alpha$  bacteriophagy belong to the R type of variant, and are indistinguishable from R variants engendered by other means. The survivors of  $\beta$  bacteriophagy are smooth in type, but how related to other smooths is uncertain. Neither variety of bacteriophage can lyse O variants. In view of Gratia's results with

antisera, Hadley regards these two types of bacteriophage as being functionally reciprocal though antigenically distinct.

Burnet (1927) has to some extent homologized certain of the many discordant results by showing that both rough and smooth variants can, under certain conditions, be produced by bacteriophagic action. Burnet has also adduced much evidence which goes to show that the irregular action of a strain of bacteriophage for different organisms is related to the possession or non-possession of a common heat-stable agglutinin amongst these. He finds that such organisms as *B. enteritidis*, *B. typhosus* and *B. pullorum*, all of which possess practically identical heat-stable agglutinogens, are affected in identical fashion by different strains of bacteriophage, *i.e.*, a bacteriophage active against one will lyse the others to approximately the same degree, and *vice versa*. With other forms in the salmonella group the degree of sensitiveness to a strain of bacteriophage corresponds roughly to the degree of relationship, as judged by the possession of common heat-stable agglutinogens. Burnet points out that a binding or adsorbing effect of dead bacteria on the bacteriophage has been demonstrated, and that this is due to some heat-stable constituent in the bacteria, since it cannot be demonstrated with cultures which have been heated to 110° C. It is therefore possible to conclude that this substance is the same as that which is concerned in the O (heat-stable) agglutinogens described by Weil and Felix. This his experiments support.

### NATURE OF THE BACTERIOPHAGE

A matter long and ardently disputed, especially amongst our Gallic neighbours. Very numerous theories and sub-theories have been put forward. The chief of these are .

I For d'Herelle, the bacteriophage is a living micro-organism, the obligatory parasite of the bacteria .

Great fleas have little fleas  
Upon their backs to bite 'em,  
And little fleas have lesser fleas,  
And so *ad infinitum* !

DE MORGAN · " A Budget of Paradoxes "

A good deal of evidence goes to show that the material is a small particulate body for which he coins the term *protobe*, against whose living nature d'Herelle will hear nothing. He points out that over this matter the division of the camps is largely an ethnological one, the more numble-brained Latins accepting the view of a living microbe, whilst the phlegmatic Anglo-Saxon, with his ingrained love for the philosophical and the obscure, favours the hypothesis of a ferment substance. It is evident that simple ferment action cannot enter into the question, since the material is reproduced on each occasion of its action and is therefore inexhaustible. There are however more complex possibilities of this nature which must be considered.

II The action of autolytic ferments; the view being that these, once introduced, set going a chain of autolytic processes in the bacteria which result in their dissolution and the freeing of the lytic substances from their interior. In this way, once the necessary environmental condition is established, the bacteria are unable to escape from it, and the process can therefore be carried on in series. This is a plausible hypothesis, though rather shrouded in mystery, and not to any great degree supported in detail. One of the major difficulties is to account for the origination of the process. Bacterial autolysis is a well-known phenomenon, but no simple set of artificial circumstances has yet been devised which will initiate this in a transmissible form. It is, moreover, an effect generally observed as the cultures age, whereas bacteriophagy is most active in young cultures.

III A theory, which is not very dissimilar, is that propounded by Bordet, who believes that a mutational change is induced in the bacteria which causes them to undergo autolysis, in this process setting free an actively lytic substance. There takes place what he terms a "hereditary nutritional vitiation" of the bacteria, which leads to their autolysis and the setting free of the lytic substance. Bordet does not appear to regard the process as an absolutely new phenomenon, but as an exaggeration of one which is normally latent, or negligible, in bacterial cultures. His views are rather overshadowed by the somewhat unfortunately obscure phase in which he summarises them, and d'Herelle

attempts their refutation on the ground that there can be no question of a hereditary transmission of lysis since bacteria-free filtrates will accomplish this. What seems to be the more correct interpretation of Bordet is that in the necessity of postulating a theory which will embrace all the phenomena of bacteriolysis, including the existence of lysogenic strains, he splits the process into two parts. Firstly, the dissolution phenomenon in a bacterial culture, which can well be due to some nutritional change in metabolism and might be conceived to yield an actively lytic filtrate on the lines propounded above. This, however, does not meet the necessity of explaining the appearance of lysogenic strains, in which the lytic principle persists in the colonies of the organisms over many generations. It is here that the "hereditary" conception is of necessity introduced. In Bordet's own words, certain bacteria, in a lytic experiment, "being more resistant, are, during a certain length of time, still capable of reproduction in spite of their producing the active principle, thus imparting to new cultures of the same microbe the same autolytic tendency" (1922). This theory has received a great deal of attention on account of the high standing of its author, who has given much study to this problem. It is somewhat involved and, like all the ferment theories, gives no inkling of the way in which such a profound mutation could be produced in the first instance, save to suggest that it is an exaggeration of a normal process. True, Bordet believes that the action of leucocytes upon bacteria may occasion it (1925), but it is clearly evident that such a source could only account for a minute fraction of the strains of bacteriophage extant and, also, that in innumerable instances the action of leucocytes or their products have no such effect on bacteria. If a nutritional alteration in strains of bacteria is produced by the bacteriophage, it is a somewhat remarkable occurrence that this unique environmental condition should produce so profound a modification with such rapidity and with such striking uniformity. Nevertheless Bordet's views, or modifications of them, are those which have found most favour.

There are, as we have stated, reasons adduced for regarding the bacteriophage as a corpuscular element and not as a material

in simple solution. The chief of these is the occurrence of the discrete plaques of lysis in surface cultures where the bacteriophage is present, for which it is hard to find an explanation upon any other supposition. D'Herelle has shown, and there is ample confirmation for this, that the number of such plaques in any given instance depends solely upon the concentration of the bacteriophage that has been added, and is independent of the concentration of bacteria, which it is difficult to conceive to be the case were the bacteriophage the product of the bacteria, which many of the theories concerning it imply. Whilst such a corpuscular conception is widely accepted for the filtrable elements, active in cultures which are undergoing bacteriolysis, it does not necessarily follow, as Hadley insists, that the influence or condition which sets the process in motion is of the same nature as the physical units by which it is serially transmitted, although it must be granted that such a view transgresses an elementary principle in logic.

One striking fact goes to show that in its action the bacteriophage is intimately bound up not only with the living state of the micro-organisms but with a particular phase of this, that of multiplication. It has been found that live organisms, washed and suspended in saline, are not susceptible to lysis. If the bacteriophage be an extrinsic corpuscular element, capable of invading the bacteria, then it is not easy to see why it should fail to produce its characteristic effect upon the bacteria under these circumstances. The fact that multiplication is a necessity for its action would therefore seem to indicate that a change is produced in the organisms in response to environmental conditions, which becomes manifested as an intrinsic alteration in succeeding generations. Such a fact is therefore more favourable to variation being at the root of the phenomenon than to a virus view of the process.

. . . . .

At the present time it is impossible finally to conclude as to the nature of the bacteriophage. The position taken up by d'Herelle is one readily defensible, since each vagary of action of the lytic principle is seized upon as yet another point of evidence

in favour of its living and adaptable nature. The position of his opponents is more difficult to maintain and easier to attack. Nevertheless they number amongst them the bulk of the more cautious, careful, and experienced bacteriologists. The matter is of intense interest and has far-reaching ramifications. It introduces a phenomenon which not only involves wide changes in cellular behaviour but which in its action is plastic and variable to a degree. The whole question of stability in bacterial species, and logically, therefore, in other cells too, is laid open and our conceptions of the nature of virus diseases are brought into the same field. The regeneration of the principle during the course of its action is not, of course, any real evidence of its living nature, since the same thing may be seen in the familiar process of autocatalysis, as for example in the hydrolysis of an ester where the acid set free serves to accelerate the reaction, or, as Bordet points out, in the production of thrombin in the clotting of blood. At the same time the discovery of the bacteriophage introduces an entirely new chapter into biology and, if the bacteriophage be not a living particle, complicates the already difficult matter of defining "life."

### **THE BACTERIOPHAGE IN THE INFECTIONS**

D'Herelle makes the widest claims for this agent as a natural therapeutic substance in the cure of disease, and as a valuable therapeutic weapon in the armamentarium of the physician. He sees, in the course of such diseases as typhoid or dysentery, a struggle for supremacy between bacteriophage and invading organisms; in the natural cure of these diseases the destruction of the bacteria by this agency; in their chronicity the period occupied in the bacteriophage in reaching a degree of "virulence" sufficient to effect this. He also believes that the rise and decline of epidemics is due to the swaying battle between bacteriophage and infecting bacteria and that, with the establishment and diffusion of a bacteriophage of sufficient activity, an epidemic is normally brought to an end. He further claims, by such means, to have treated successfully cases of dysentery, typhoid and other

diseases and, in the field, to have controlled and stamped out epidemics of avian typhoid and the hæmorrhagic septicæmia (barbone) of bovines "The beginning of an epidemic is marked by the diffusion of the causal bacteria; its end by the diffusion of the bacteriophage, virulent for this bacterium" (d'Herelle).

The accuracy of these statements has been investigated by Topley, Wilson and Lewis (1925), in series of carefully controlled experiments with mouse typhoid (*B. aertrycke*) infection. Introducing a bacteriophage of marked activity into the drinking water of the mice, during an early period of the infection, and making daily quantitative estimations of the excretion of *B. aertrycke*, they found no difference whatever in this respect between the mice so treated and the control animals, although the bacteriophage was frequently demonstrable in the fæces of the former. Neither did they find that any influence was exerted upon the mortality from this infection. They further found that the administration of the lytic filtrate, simultaneously with the infecting organism, to groups of mice did not influence the frequency or course of the subsequent infection. In experimental epidemics of mouse typhoid subcutaneous inoculation with the bacteriophage also failed to modify in any way the normal course of the event (Topley and Wilson, 1925).

The claim is made by d'Herelle that by injection of cultures lysed by the bacteriophage two types of immunity are established: firstly, an immediate resistance, due to the presence in the body of the bacteriophage, which lasts only as long as this substance can be detected and is therefore fleeting and, secondly, a more slowly developing solid type of immunity, which is simply a result of the injection of the products of the killed bacteria and is the type of antibacterial acquired immunity abundantly familiar to bacteriologists.

It was a finding of Krestownikowa and Gubin (1926) that when the bacteriophage is injected into the body it appears in all the tissues within a few minutes, but is no longer detectable after six and a half to eight and a half hours have elapsed. Consequently any possible active immunity which is conferred upon the healthy body by its presence must be very shortlived.

Topley, Wilson and Lewis have also investigated the question

of these two types of immunity being in play in the case of mice infected with *B. aertrycke*. They found no evidence of any immediate immunity, either from the injection or ingestion of a bacteriophage lytic for this organism. Ingestion produced neither early nor delayed immunity, but injections of the lysed cultures produced a satisfactory degree of immunity after the lapse of the ordinary latent period of several days

In connection with the latter point d'Herelle states that lysed cultures of *B. dysenteriae* (*Shiga*) become non-toxic after an interval of a month or more and can then be used for ordinary vaccine prophylaxis, a matter which, as is well known, is usually hedged about with great difficulties on account of the well-known toxicity of this bacillus and the severe reactions which it occasions.

The claims for a therapeutic value for the lytic agent should have been speedily confirmed if they were well founded, since they are sufficiently revolutionary to attract attention and the method is one easy of application. In general it may be said that no such confirmation has been forthcoming. Except in South America, from whence some positive results have been reported, the agent seems therapeutically impotent in dysentery, in which its action should be especially well marked. In one report, that of Fletcher and Kanagarayer, a preparation of bacteriophage for the treatment of Flexner dysentery in Malaya was supplied by d'Herelle himself, and even here the results were completely negative

Certain favourable results with bacteriophage treatment have been reported in localised septic infection (*e.g.* Gratia, 1922), but here, as in other instances in which there is direct and intimate contact between the invading bacteria and therapeutic agents of this nature, it is not easy to exclude the influence of other bacterial products, which in certain cases have been found to exercise a markedly inhibitory effect (Besredka). In the case of genito-urinary infections with coliform bacilli the method has had a fair trial, and in the opinion of some workers is effective. Larkum (1926) has made some interesting observations in this respect, and finds that in most acute urinary infections a bacteriophage is to be detected in the urine at some period, the principle, in his

experience, came and went in the urine in inexplicable fashion. In the acute cases, when present, it was found along with lysogenic strains of the infecting bacillus and did not persist in the bladder in the absence of such strains, which were preceded and succeeded by the ordinary lysable but not lysogenic varieties. In chronic cases of the disease it is perhaps significant to note that he found the organism usually present to be resistant to lysis.

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*THE BACTERIOPHAGE*

FLETCHER and KANAGARAYER    Bulletin No. 3    Institute for  
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(Only the papers referred to in the text are given above. The literature on the subject is very large )

## CHAPTER V

### EXPERIMENTAL EPIDEMIOLOGY

[In the abstract of recent work in this field, which follows here, the writer wishes to acknowledge his especial indebtedness to the work of Topley, whose papers he has very extensively drawn upon in its preparation.]

Increasing knowledge of bacteriology and bacterial distribution, and analyses of the relationship of host and parasite, have served to indicate the very great complexity of the problems not merely of the epidemic but also of the endemic state, and of sporadically occurring disease. The attack which has been made upon these of recent years, especially at the hands of Topley, in Great Britain ; Flexner, Amos, Webster and Pritchett, in America ; and Neufeld and Lange, in Germany, has been directed chiefly towards the elucidation of the conditions underlying the epidemic, upon which same conditions, with certain modifications, the other forms of disease incidence are doubtless also dependent. It must be remembered, at the outset, that the spread of disease and the spread of infection, although occurring together, are not the same thing, though infection is most obviously made manifest by disease. Studies upon the carrier state have shown that an infection may be widespread amongst a population with but a few cases of disease showing themselves. The matter is well illustrated in epidemic cerebrospinal meningitis, in which under certain circumstances the carrier rate in collections of individuals may be a high proportion of the total population, and yet the cases of disease may be relatively few. As Arkwright points out, the epidemic here is an epidemic distribution of meningococci amongst the population in question. There are good reasons to believe

that similar circumstances may apply in other infections, though they may not be so readily demonstrable as in the case of cerebrospinal meningitis, and that in general the distribution of a causal organism is far more extensive than is indicated by the prevalence of its specific disease. In the epidemic spread of a disease a whole series of reactions between host and parasite may occur, which depend upon the interplay of at least two main factors, microbial virulence, using this term in its widest possible sense, and the resistance of the host. The possible outcomes of such interrelationship are various. To quote Topley "We may suppose that the primary interaction of the pathogenic bacteria and the susceptible hosts will lead to fatal infections, to infections clinically recognisable as cases of disease, to atypical infections involving some recognisable departure from health, and to latent infections or the carrier state, in which the host will harbour the parasite over long intervals of time without any sign of disease." It has been the object of the experimental epidemiological studies, with which we are for the moment concerned, to sift as far as possible, under the conditions of a controlled experiment, the various factors which condition such varied results and, by reducing the number of variables in the way possible only in laboratory experiments, to ascertain what is the exact bearing of each of them upon the problem in hand. It is necessary to repeat the warning which has been uttered by all workers in this field, that direct deductions bearing upon general epidemiology should be made only with the greatest caution from these laboratory experiments, since human epidemics are incomparably more involved than are those set up in the experimental institute. There are, however, lines of broad agreement between clinical studies and those of experimental epidemiology which are hopeful indications for further progress in this field of research.

The method generally followed, by all workers, has been to introduce infected animals amongst populations of mice and to observe the course of the ensuing mortality. The conditions of the experiment may be varied from time to time so as to study such factors as information is desired upon. Topley has worked with *salmonella (aertrycke)* and *pasteurella* organisms, Webster with a

similar salmonella, and a respiratory (*B. lepi-septicum*) infection of rabbits. Webster attempted to control individual variation amongst his mice by using pure line strains, which had been inbred for at least five years, and to avoid variations in dosage by the intragastric injection of the bacteria. He believes that by such means he has been able to obtain accurate knowledge of the extent of such variation amongst the subjects of his experiments and, by artificial selection, to breed strains in which the average resistance is greater or less than normal. Topley has dealt with unselected groups of mice, so that in his experiments the individual factor is probably subject to a wider range of variation than is the case with Webster's.

### THE CURVE OF THE EPIDEMIC

When an infection, such as *B. enteritidis*, is introduced into a population of mice by the method of adding a few infected individuals the course followed is that shown in Fig. 6, which gives the results of ten such epidemics observed by Topley over periods of from sixty to eighty-four days. It will be seen that after an initial period of lag the curve of the epidemic courses steeply downwards; but that instead of proceeding to the extermination of the little communities, it tends to flatten out and the epidemic to come to an end. Deaths become fewer and fewer, and in these cases had almost ceased to occur by the end of three months. Topley also observed that the separate epidemics tended to have an individual character, some proceeding rapidly, others more slowly and with fewer deaths. This point is brought out in the chart. The epidemics which pursued a milder course were the ones which developed more slowly *ab initio*.

There are therefore at least three phases in an epidemic which are open to more critical study: pre-epidemic phase, the epidemic phase, and the post-epidemic condition.

**The Pre-epidemic Phase.**—In this period, which extends from the time of introduction of the infection to the outbreak of the main epidemic, it has been found that isolated and scattered deaths occur amongst the susceptible animals, but that the existence of a

long latent period before the outbreak of the epidemic proper is a constant occurrence. This period in the experiments we are now considering was from one to two months or more. The results obtained are charted in the next two figures

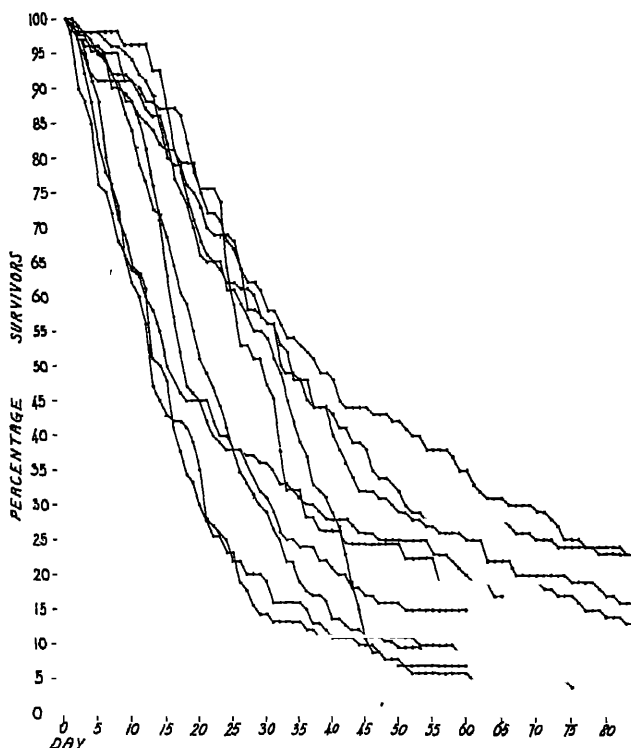


FIG 6—The daily percentage of survivors in ten mouse populations artificially infected with *B. enteritidis*—(Topley.)

Fig 7 is for the case of *B. enteritidis* infections in mouse populations which consisted of three infected individuals only on the day the experiment started. Three additional mice were added daily in the first two cases and irregular batches were added in the third. In each case the mouse population was well over 100 before the onset of the main epidemic, only the

commencement of which is shown in the charts, from which the original infecting mice are also excluded. A similar result was

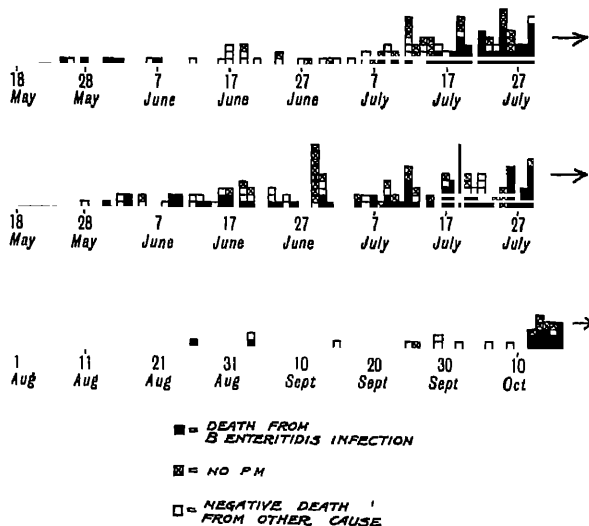


FIG 7—Showing the incidence of deaths, and their causes, in populations of mice in which the infection was introduced on the date shown on the left-hand side (The arrow indicates the continuance of the epidemic, which is not charted) —(Topley.)

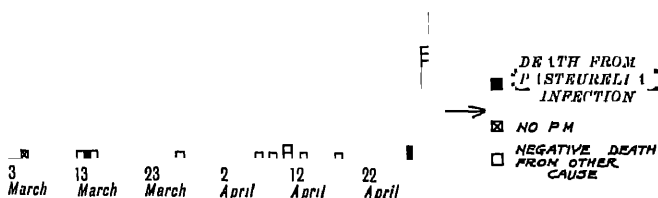


FIG 8—As in Fig 7, but showing the commencement of a spontaneous pasteurella infection in a group of 120 mice. The first case occurred on March 14th, but the epidemic did not break out until April 27th —(Topley.)

noted in a spontaneously occurring epidemic of pasteurella infection, in a group of 120 mice which had been collected together for another purpose (Fig. 8) Those who are conversant with

Defoe's writings upon the plague will recall that he comments upon an exactly similar occurrence at the commencement of the terrible outbreak of 1664. In the course of these investigations Topley repeatedly noted that during this pre-epidemic period there was an increase in the general mortality from all causes amongst his mice. The discussion of the significance of these findings we will leave for the moment.

**The Epidemic Phase.**—The course of an epidemic amongst a fixed population of mice has been dealt with already. An investigation which leads to more significant results is that concerned with the fate of susceptible animals which are added to a population already infected, but in which the disease has died down and, as tends to be the case, a stage of equilibrium has been reached. It is found that this procedure always results in a fresh outbreak of the disease: a recrudescence of the epidemic which involves not merely the newcomers but also the old inhabitants, who up to this time had survived the infection which was present in their midst. This latter observation is particularly significant. By such means an epidemic can be kept going over very long periods, apparently indefinitely. When this is done, and increments to the population regularly made, it is found that the mortality does not maintain a regular level, but waxes and wanes, so that a series of peaks occur upon the mortality curves denoting the periodic exacerbations of the epidemic. These periods of more intense mortality are preceded by periods of quiet, in which the population gradually increases from its regularly added increments until the concentration of animals has risen considerably, when the circumstances become suitable for a fresh flare-up of the malady. This regular series of epidemic waves is best seen where the additions to the population experimented upon are made slowly; where they are more frequent, and a high concentration of mice is maintained in the cages, the periodic rise and fall of mortality is much less well marked. The quiet intervals in the former cases are often quite prolonged; in one experiment of Topley's, which lasted for over two years, a period of ten months intervened between one series of deaths from a *pasteurella* infection and the succeeding one.

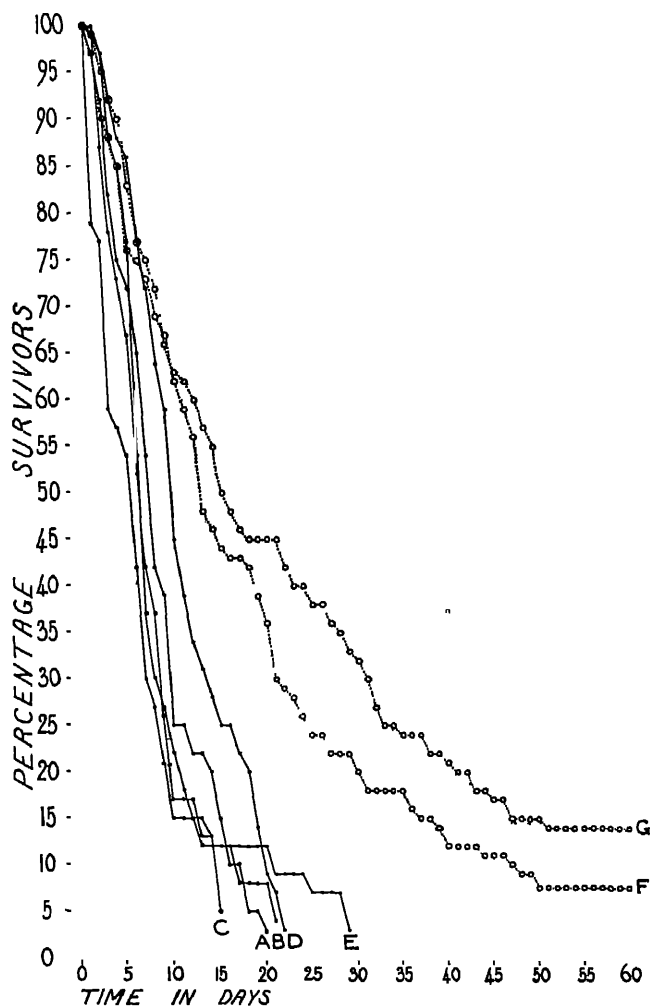


FIG. 9.—Showing the self-limiting nature of an epidemic in a fixed population (curves G and F), and the tendency to extermination prevailing where fresh susceptible subjects are constantly added to the population (curves A—E).—(Topley.)

The effect of adding fresh susceptible individuals to a population in which an equilibrium is being arrived at between the infecting

agent and the hosts is, as we have stated, to cause a fresh series of deaths, which involve not only the newcomers, but also the survivors, so that from the point of view of the latter this immigration is disastrous. Only by such inter-reactions do these epidemics lose their self-limiting character and tend towards extermination of the infected population. It has already been shown (Fig. 6) that the ordinary epidemic in a fixed population has a self-limiting character. Fig. 9 shows the lethal effect of a continued introduction of fresh subjects. Curves G and F are of the self-limiting type previously figured, and refer to epidemics which were allowed to pursue their normal evolution in two series of 100 mice; at the termination of this experiment the infection (*B. aertrycke*) had produced a mortality of about 85 to 90 per cent. in the two populations. In the groups making up the other curves (A to E) daily additions of fresh mice were made, and here it is obvious that the tendency of the process is towards extermination, only 8 to 5 per cent surviving at the end of the experiment.

If we review these fundamental experimental findings, we note that for an epidemic to occur, a certain relationship of host to parasite is necessary, which is not immediately established when the two are brought into contact. It seems clear that the infection is disseminated for some time prior to the epidemic rise in mortality, and this indeed is proved by Topley's studies upon the excretion of the infecting organisms in *B. enteritidis* infections. For the epidemic to occur, some as yet unanalysed factors intervene, to upset the temporary equilibrium which appears to be established between host and parasite. One such factor, apparently essential for a good epidemic, has been found by Topley to consist in the maintenance of the population at a fairly high numerical level. In small groups, after a few cases of the disease have occurred, the infection tends to die out.

A series of experiments which bears upon this shows the result of splitting up an infected population into a number of small units. Fig. 10 illustrates the result of such a dispersal, carried out in the pre-epidemic phase of a *B. enteritidis* (*aertrycke*) infection

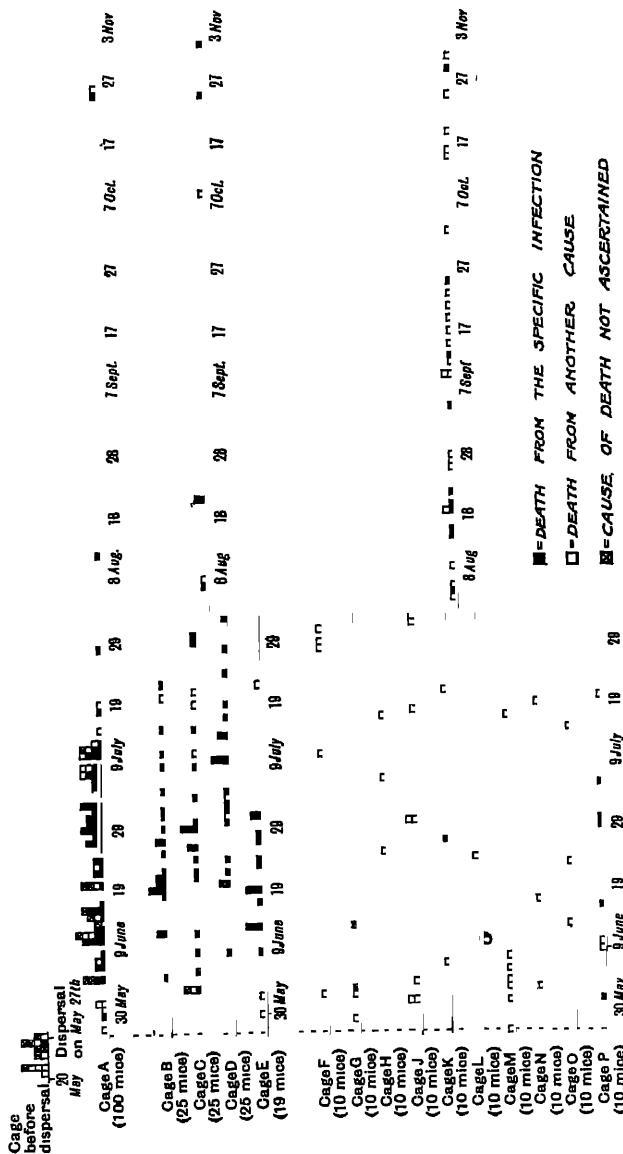


FIG 10.—Three hundred mice had an infection (*B. anthracis*) introduced into their midst. Eleven days later they were split up into one population of 100, four of 19–25, and ten of 10. The chart shows the course of the infection in these variously-sized populations and the effect at a later stage of their reconstitution into larger batches—(Topley)

of eleven days' standing. It is evident that, within the time limits of the experiment, such a procedure produces a notable decrease in the mortality, which furthermore bears a direct relationship to the degree of the dispersal. A second point which emerges, is that upon reconstitution of the small colonies into a single population fresh cases of the disease will occur, but nevertheless the total mortality remains lower than in the control series. This point will be further referred to anon. In similar experiments, in which the splitting up into small units was effected in the epidemic instead of the pre-epidemic phase, the same effect was observed but was not so marked. Here the self-limiting tendency of the epidemic appeared earlier in the dispersed than in the undispersed animals, and the percentage of survivors was consequently higher in the former than in the latter.

The possible factors which govern the ultimate onset of the epidemic wave are : dosage of the virus, virulence of the organisms, and host resistance, all being considered in their widest aspects. In the case of resistance, we are not concerned here with the resistance of individuals, but with the common level of the threshold of the whole population which is exposed to risk and from which individual variations may be considerable. Since, however, in the infections here dealt with, the effective ability of the parasite to cause disease and death is relatively high under all conditions, it can hardly be assumed that variations in host resistance condition the rise of the epidemic curve after its initial delay. Dosage and virulence are matters which are extremely hard to assay, and into a consideration of them enter many obvious factors which it seems almost if not quite impossible to control in individual experiments. One main and evident factor lies in the close contact of large numbers of susceptible animals, and Topley shows that in the pre-epidemic phase, in the case of *B. enteritidis* infection, a marked rise in the excretion rate of the organism occurs amongst the exposed animals, a rate which is already falling when the epidemic curve is on the up grade. There is, therefore, every chance that in this period a somewhat intensive dosage of bacteria will be ingested by the animals at risk, and it is extremely probable that infection and reinfection, merely by increasing the dosage of the

organisms, play an important part in the rise in morbidity which follows so rapidly. It is no doubt this factor also which plays a prominent part in the fatal infection of the old survivors, which is a constant feature of any fresh outburst of epidemic disease in a population which has reached equilibrium. With regard to virulence, Webster is of the opinion that little variation in this occurs in the course of a mouse-typhoid epidemic. Topley, on the other hand, concludes that significant alterations in virulence do occur, and that these may be produced by passage. What part is played by this, and whether or how it reacts upon the animals in the course of absorption, excretion and reabsorption, it is impossible to say. It is also pointed out that "virulence" includes other properties than toxicity, such as ability to live and pullulate on mucous surfaces, and to invade the body, which may be all-important in naturally occurring infections, but which we have no ready means of evaluating.

In regard to the established epidemic, and its periodically recurring waves, some such similar factors must be at work here: at one moment the organism betrays a maximum lethal activity, and the mortality rises, whilst at a later period there seems to be a temporary ascendancy on the part of the hosts and the mortality rate falls and may even become nil. As fresh susceptible individuals are added and accumulate the cycle of events is repeated. Both Topley and Amos have observed that this does not hold in the same regular way where large batches of mice are added at irregular intervals to the infected population. Sometimes a rise in the death rate is the result, but sometimes no effect at all is observed. It is suggested that the result depends upon the exact stage of the balance between host and organism which exists at the time the addition is made. Sometimes this may be at an equilibrium and sometimes, as the previous experiments have shown, it may be temporarily disturbed in one direction or in the other.

**The Post-epidemic State.**—In the experiments already described we have noted how a simple epidemic tends to be self-determined, and to come to an end after producing a certain mortality in the population. The survivors are therefore evidently possessed of a

superior degree of resistance than the rest of the population ; and the question arises as to whether they have developed this, or have been endowed with it from the beginning of the experiment

If we turn back to the experiment illustrated by Fig. 10, we shall see that where the small groups into which the population at risk have been split are reconstituted, the mortality still remains low. In this case the effect of concentration of a number of individuals in the vicinity of infection is not the usual one, and it may therefore be inferred that these individuals have become in some way immune. They do not react as unselected batches of normal mice react under similar circumstances , and it is therefore suggested that their immunity is acquired and not innate. The parasite is still present amongst them, for the introduction of fresh unselected mice will, as we have previously seen, give rise to an epidemic outburst, fatal alike to newcomers and survivors, but it is evident that in these old stagers a state of equilibrium between parasite and host has been reached.

That this is the case has been directly demonstrated by Topley by the examination of the survivors from various epidemics , 60 to 100 per cent. of the mice which have survived epidemics of *B. aertrycke* infection may be shown to harbour the organism in their spleen, although to outward appearance healthy In these cases, therefore, survival is associated with a definite non-effective infection, and immunity is established at the expense of some sort of symbiosis , a phenomenon we are abundantly familiar with in clinical medicine in the cases of tuberculosis and syphilis. It must be assumed, however, that at a later stage this is ended at the expense of the parasite, and it is doubtful if such a conception can apply to more than a limited number of infections. Webster believes that it is selection which is particularly prominent in eliminating the naturally susceptible individuals in the course of an epidemic and favouring the survival of those endowed with greater natural resistance Undoubtedly such factors must enter into any epidemic condition, but the extent to which they exist, and the importance of the part they play, is a much more doubtful matter, and Topley does not appear to regard it as being as potent as Webster does. He, on the other hand, regards the survival of

certain animals as being clearly due to their active immunisation in the course of the epidemic.

In favour of this view are the findings of the organisms in the surviving animals' tissues and the further fact that immune bodies can be frequently demonstrated in their blood. Amos found agglutinins for *B. aertrycke* in thirty-seven out of fifty-six mice which had survived an epidemic of this infection, and Topley has had a similar experience. In one of his experiments, out of a population of 121 mice exposed to *B. aertrycke*, and yielding fifty-five survivors, only six of these failed to show some evidence of the infection. Wide dissemination of the infecting agent in these experiments, and the increased average resistance shown by the survivors, seem to point quite clearly to the fact that active immunisation plays an important part in determining the fate of those members of the community which survive. In experiments conducted upon pasteurella infections, Topley and his collaborators reach the same conclusions; and they further find that this immunity is related both to the length of time the animals have been exposed to the infection and the degree of severity of the latter. Of these two the first factor seems to be the more important.

Since an acquired immunity in the population at large has the natural effect of bringing an epidemic to a termination, it is interesting to ask what the result of this will be if it is induced at an earlier period, a procedure towards which hygienists and epidemiologists so constantly strive in both human and animal medicine. The experimental animal epidemics lend themselves to a more exact study of the results of such procedures than do field experiments in man, and have been utilised in this way both by Webster and by Topley. The result of infecting populations of immunised, unimmunised, and mixed mice was in every case a marked reduction in mortality in the populations in which all the mice were immunised or which contained considerable proportions of immunised animals. In another experiment of Topley's the effort was made to find out what was the effect of immunising the animals at different stages in the progress of an epidemic. The results, which are extremely interesting, are

summarised below. Four groups of 100 mice were utilised, and they were kept under observation for eighty-four days.

Group A.	Mice which had received two doses of vaccine before exposure to infection	71 per cent. survived.
„ B	Mice receiving one inoculation on the fourteenth and one on the twenty-first day of risk	52 „ „
„ C.	Mice receiving one inoculation on the twenty-eighth and one on the thirty-fifth day	43 „ „
„ D.	Mice receiving no inoculation	51 „ „

It is evident that only when the inoculation was completed before exposure to infection, and such immunity as developed well established, was any benefit conferred upon the animals. A study of the curves of the mortality further shows that in Classes B, C and D the epidemic evolved in similar fashion, and that only in Class A was there any significant difference. Here both the evolution and progress of the epidemic were slow, and its course represented in exaggerated form the type of slowly developing and mild epidemic which sometimes, though exceptionally, occurred in unprotected communities in these infections.

In considering the above findings, in the light of our experience and practice in human disease, Topley utters a warning against pressing the deductions too far. This we would emphasise, for the opportunities for infection and reinfection are at a maximum amongst the confined communities of animals dealt with, and their habits totally unlike those which prevail amongst civilised human subjects under any circumstances. One factor, whose importance is clearly shown in these investigations, is that of overcrowding. Another outstanding consideration is the evil effect of the constant reintroduction of fresh susceptible individuals into a community. As far as experimental evidence goes, the little Derbyshire community of Eyam, who rigorously isolated themselves in their village in the time of the plague and let it wreak its fury upon them, were serving the larger community in a way later knowledge cannot suggest any improvement upon. Had they

dispersed, like Boccaccio's company or Defoe's wandering bands, they might have increased their individual chances of survival, but, had the infection been present amongst them, by intercourse with others they would have disseminated their disease through the countryside. At all events, by their self-sacrifice, they were spared the definitely increased chances of infection which could have resulted from the constant immigration of susceptible and well-meaning newcomers from without.

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## CHAPTER VI

### CALMETTE AND B.C.G.

*" . . . après le vent de folie qui a poussé tant de nations, soi-disant civilisées, à s'entre-détruire, l'œuvre de paix réparatrice imposera, plus que jamais, aux hommes de bonne volonté, le devoir de travailler à la sauvegarde des innombrables vies humaines que fauche prématurément la tuberculose."*—A. CALMETTE.

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It is a commonplace in the experience of all pathologists that an active immunity against tuberculosis may exist. The frequency of obsolete tuberculosis, discerned only at post mortem ; the superior resistance displayed by the city dweller, in which these findings are numerous, by contrast with the greater susceptibility of his more robust-looking country cousin ; the general curability of surgical tuberculosis, better realised to-day than it was a couple of decades ago—all of these point in the same direction. On the experimental side the Koch phenomenon indicates, *inter alia*, a marked reaction on the part of the infected and partially immunised animal against a fresh infection, whilst the serum antibodies are similar evidence of the immunity developed by the infected subject.

Attempts to establish this immunity in man have been made almost exclusively in connection with the already seriously infected, and in this condition have yet to be developed as generally practicable and useful therapeutic measures. On the other hand, experiments upon healthy animals, directed towards the establishing of a condition of resistance, have been the object of innumerable researches. The vast majority of these have been devoted to the development of immunity by the injection of tubercle bacilli, more or less modified, or of their products. The

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bacilli have sometimes been simply killed by heat, and by this means a definite though not very satisfactory immunity has been obtained. Other investigations have been directed towards finding an antigenic and immunising fraction by means of the chemical decomposition of the bacterial bodies. The fatty and lipid substances have been found to be non-antigenic, whilst the protein residue, though definitely antigenic and conveying in certain cases a sensible degree of immunity, has not given results which are of very great value, or which are any better than those produced by the dead bacillary bodies alone.

In yet other cases workers have sought to modify the toxicity of the dead bacilli by less energetic means than chemical decomposition. Chlorine, sodium fluoride, sodium oleate, neurine, choline, urea, glycerin, galactose, lactic acid, chloral hydrate, ultra-violet light, and proteolytic enzymes, have all been employed with this end in view; as have also bacilli sensitised with immune serum. In general, it would appear that the methods which cause the least damage to the bacterial structure are those which give rise to the most efficient antigen, and in some cases the reports show that a high degree of immunity has been conferred upon experimental animals by such modified bacilli.

It is probably a realisation that the most satisfactory method of inducing artificial immunity is that in which an attenuated living virus is employed—the true Pasteurian method—rather than any injection of dead organisms, that has led to numerous attempts being made to effect anti-tuberculous immunisation by such means. One of the earliest large-scale investigations of the practicability of such methods was carried out by a French veterinary commission under the supervision of Rossignol and Vallée. The vaccine employed was that of von Behring, Romer and Ruppell (bovo-vaccine).

The essential of this method was that cattle were inoculated intravenously with old laboratory cultures of *human* tubercle bacilli, which had been dried *in vacuo* and which possessed only a slight degree of virulence for the guinea-pig. Twenty-one animals were inoculated in all and these were submitted to various tests of their immunity three months later.

The results of this investigation may be briefly summarised :

- (a) One animal died fifty-seven days after inoculation ; a second was killed nine months later. Neither of these animals showed any tuberculous lesions and their lymphatic glands failed to infect guinea-pigs.
- (b) Six of the animals were tested by the inoculation of 4.5 mg. of virulent bovine bacilli intravenously. When killed three months later four were free from visible tubercle, but their bronchial glands produced tuberculosis in guinea-pigs ; two showed slight tuberculous lesions of the bronchial glands. The control animals showed massive generalised tuberculosis and three of them had died before the experiment terminated.
- (c) Seven animals received subcutaneous test-inoculations with the virulent bovine bacilli. Four developed trivial local lesions with slight involvement of the adjacent glands, and three showed no local lesion. Nevertheless, in all, the lymphatic glands draining the injected area were shown by guinea-pig inoculation to harbour virulent tubercle bacilli.
- (d) Two of the immunised animals were kept in close confinement with other cattle suffering from advanced pulmonary tuberculosis. Both were killed after a year of this existence : one of these showed extensive tuberculous lesions, the other a single focus in one tonsil. Controls became heavily infected.
- (e) Four animals were not tested with the others, at the end of three months, but were utilised to demonstrate the duration of the immunity.

Two were inoculated intravenously a year later. One of these died in forty-seven days (no protection) ; the other lived and appeared healthy, but on being killed a year later showed generalised tuberculosis. Two others were kept for two years and then placed for thirty-three days in close confinement with tuberculous animals. Only one of these contracted tuberculosis.

This experiment, though an old one, is instructive in showing

the possibility of inoculating bovines against tubercle by means of the human type of bacillus, and, further, that the attenuated living form of this organism is seemingly harmless for these beasts and confers a very definite degree of immunity upon them. The immunity, however, is only transitory, and was disappointingly ineffective in the case of animals exposed to natural, though intense, contagion. The trial of von Behring's bovo-vaccine in the hands of others has given similar results, the maximum degree of immunity being developed in about three months and its duration being short. For this reason it is not a procedure of great practical value, and, moreover, since living bacilli of the human type, even if attenuated in virulence, are introduced into the animal and subsequently appear in its secretions, it is not a procedure to be used in dairy cattle. S. Griffiths has shown experimentally that this is no mere theoretical objection even when the vaccine's use is restricted to the young calf.

One of the points brought out in the above experiment, and a point of no little importance, is that the resistant state is not necessarily associated with an ability on the part of the organism to destroy completely the invading tubercle bacilli, even though these appear to make little or no progress in the tissues. What happens, to some extent, is that they are prevented from setting up their accustomed lesions, but, nevertheless, remain living and potent in certain situations, notably in the lymphatic glands, where in another connection Calmette and his associates (1921) succeeded, in the case of the dog, in demonstrating them in a latent condition three months after they had been injected. It may therefore happen that in experiments of this sort the virulent bacilli continue to exist in the tissues in a dormant state until the animal's artificial immunity wanes, when they become capable of springing into full activity and setting up the ordinary lesions of the disease.

A somewhat similar preparation, but composed of attenuated bovine bacilli, was brought out by Koch and Schutz and put upon the market in Germany, in a commercial form, under the name of "tauruman." The bacilli in this preparation are virulent for guinea-pigs and its employment seems fraught with considerable

risks, which also apply to the attenuated bovine vaccine suggested by Theobald Smith (1908). All such methods of inoculation of the bovine species with virulent living organisms are open to untoward effects from the presence of such organisms in the tissues, and the immunity has in no case been shown to be of any but short duration.

✓ The vaccination of man against tubercle, by means of organisms of lesser virulence than the human bacillus, is a daily occurrence through the ever-present evil of infected milk. Although the disastrous effects of this upon infant health are in certain respects well recognised, there is another and a more subtle aspect of the matter which raises questions as yet unsettled, viz. . How do individuals stand who, escaping the gross visible pathological results of such an infection, are, nevertheless, invaded by the bovine bacillus? By analogy, and by reference to the general principles of artificial immunisation, they may well be better equipped against the risks of later infection with human bacilli, and pulmonary involvement, than are those whose tissues have at no time harboured tubercle bacilli of any type.

In the course of his numerous researches upon tuberculosis, and in accordance with his well-known individual views about the route of infection in man and in animals, Calmette found that the vaccination of cattle against tuberculosis could be carried out equally well by the digestive tract as by parenteral routes, and he also put forward a claim that in respect of duration of the immunity this route offered advantages over the latter. The view is urged that active immunity against tuberculosis, whether it result from artificial procedures or from natural infection, is active only so long as living tubercle bacilli, or actual tuberculous lesions, are present in the body. With this belief in mind, Calmette set about finding a form of vaccine which would be absorbed by the natural channels and persist in the body over a long period, thus providing a prolonged immunity. The organism envisaged should be resistant to phagocytosis but incapable of setting up the lesions of tuberculosis.

Calmette and Guérin claim to have discovered such an organism in a bovine type of tubercle bacillus which has been cultivated for

long periods in the presence of bile. They state that the organisms become modified, both in morphological characters and virulence, so that after about seventy passages on such a medium a young ox will tolerate an intravenous injection of 100 mg. of such a culture, whereas a control beast, injected with 8 mg. of the same strain grown upon ordinary potato medium, succumbs in about a month with acute miliary lesions. The animal receiving the culture from the bile-containing medium suffers only from a short febrile illness, which disappears in fifteen to twenty days, and is not associated with any development of tubercles: it results nevertheless in an abundant production of antibodies. Animals treated in this way develop a high degree of immunity towards test intravenous injections of virulent bacilli. Similar results were obtained in the protection of the guinea-pig, both when the vaccine was introduced directly into the blood stream or when it was given by way of the alimentary canal. The animals, after a latent period of some twenty days, suffered from a sharp illness with general swelling of the lymphatic glands. The symptoms then disappeared and the guinea-pigs appeared to be none the worse. If they were killed during the acute phase of this illness the bacilli could be detected in the lymphatic glands and other organs, but the lesions typical of tuberculosis were absent. The phagocytosis of the organisms by large mononuclear cells was seen to be in active process. When at a later period the immunity of the animals was tested by Calmette's method, which consists in instilling a drop of a virulent culture into the conjunctival sac, a procedure which leads to a progressive tuberculosis with gross implication of the cervical glands and which it is claimed more closely simulates the naturally occurring disease than infection by other methods, the treated guinea-pigs remained healthy over two or three months—in which time the controls had perished with advanced cervical and general lesions. When killed the guinea-pigs thus treated showed slight enlargement of certain cervical glands, which proved to be due to an excessively chronic and fibrous form of tuberculosis, in which the bacilli could only rarely be seen.

The method was put into practical use in the immunisation of

cattle, and is at the present moment being extensively investigated. Calmette insists that the immunisation shall be commenced in the new-born calf by the subcutaneous injection of 50–100 mg. of B.C.G., and the inoculation repeated annually. Except for the peculiar features of the culture employed, this does not appear to differ appreciably from other of the many methods of bovine immunisation which have been proposed.

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The definitely immunising property of these modified living bacilli, and their apparent complete innocuousness, suggested to Calmette the possibility of applying a similar form of immunisation to the human subject and, in particular, to children. With this object in view it was proposed that certain preliminary experiments should be carried out upon apes, in a region in which they were not exposed to the multiple risks of tuberculosis which affect these animals in temperate countries, and which so frequently vitiate observations made upon them in regard to this disease. This was done by Wilbert (1925), at the Pasteur Institute of Kindia (French Guinea), where arrangements are in being for the breeding and maintenance of large stocks of simians for the needs of French medical research. The experiments, which were carried out on a generous scale upon divers species of monkeys, were designed to show :

- (1) If the vaccine "B.C.G." (Bacille Calmette-Guérin) were toxic or dangerous towards these animals.
- (2) If it protected them against tuberculosis.

The results showed, in respect of the first query, that the subcutaneous injection of 50–100 mg. of the living B.C.G. gave rise to no harmful result. A local reaction followed the larger dose, but not the lesser one, and the animals which were killed at a later date, or which died of intercurrent disease, in no case showed any signs of tuberculosis. The dosing of the animals by the mouth with 250 mg of the culture, divided into five doses of 50 mg. given every second day, was likewise found to be completely innocuous.

To elucidate the second of the two problems animals were inoculated subcutaneously with a single dose of 50 mg. of the culture, or else were given 250 mg. by the mouth, divided up into five doses in the way just mentioned. The organisms which were employed at a later date to test the resistance of the inoculated animals were human and bovine cultures of normal virulence. It may be said at once that no appreciable difference was detected between the action of these two varieties of virulent bacilli.

The effect of the vaccination was in most cases tested by prolonged and close cohabitation between the vaccinated animals and animals which had been artificially infected with tuberculosis; control, normal, animals being always introduced into the cages at the same time. Nineteen monkeys were immunised by B.C.G.; 4 by ingestion and 15 by inoculation. Twenty animals were artificially infected with tubercle, either by the mouth or by injection, and were caged with the immunised ones, whilst 20 others were used for control observations. The experiment lasted over a period of about sixteen months. By the time this period had passed, and the results were reported, all the inoculated and control animals were dead, 38 of them of tuberculosis and 2 of intercurrent maladies. Of the 19 animals treated with B.C.G. 6 were alive and seemingly in good health; the rest had perished of accidents and intercurrent infections. None of the last-mentioned animals showed any signs of tuberculosis at post-mortem examination.

In taking stock of the results of this extensive experiment, the high mortality of the treated animals from "intercurrent disease" is striking. It should be pointed out, however, that their tenure of life under the experiment was very considerably longer than that of the infected animals, or of the controls, most of whom succumbed in three to five months. The apparently high incidence of death from such causes may therefore be considerably discounted. It may be further added that, in a paper published in 1928, Calmette states that the immunity which was set up in these animals in 1923-24 was still effective.

As a result of these investigations it appeared plain to Calmette

that as far as animal experiment would go the attenuated strains of bacilli might be administered without risk and with good prospects of some immunity resulting from their absorption. In view of the high infantile mortality from tuberculosis in Paris, where 28 per cent. of all deaths in hospitals during the first year of life, and 26 per cent. during the second, are due to this disease, it seemed to him that the application of such a method to human infants was justifiable. Calmette at that time considered that the method, if applied at all, should be restricted to children at a very early stage of their existence, at which they had not yet contracted those tuberculous infections which are generally believed to be the common lot of city dwellers. Applied at a later stage, at which the tuberculin test has become positive in a high proportion of cases, it seemed to him that dangerous allergic reactions might be set up, with all the well-known risks of stimulating into activity slumbering lesions and the freeing of latent but virulent organisms. Amongst those of all ages and social conditions who run the risk of tuberculosis the most perilous lot would seem to be that of the children of tuberculous parents, and of these particularly young infants the mothers of whom are the victims of active tuberculosis. Figures collected by Calmette from various sources show that the mortality from tuberculosis in such infants, during the first year of their existence, is variously estimated at between 20-70 per cent., and in France is on an average about 25 per cent. annually.

In July, 1921, Calmette began to attempt the immunisation of such infants against these very great risks by means of the oral administration of B.C.G. A first experiment showed that an infant of three days old tolerated three successive doses of 2 mg. of the vaccine without mishap and developed normally. Encouraged by this result, Calmette treated 217 newly-born infants during 1922, and later reported that the results were favourable. In 1926 he reported the following more extensive results, which we have extracted from his papers

Total number of infants treated with B.C.G. = 5,183

Total number traced = 1,817

*Condition of the Cases Traced*

Vaccinated 12-18 months previously . . . : 564

Alive	{ Contacts . . . . . 281 }	. . . . . 519
	{ Non-contacts . . . . . 288 }	
Dead	{ Non-tuberculous disease . . . . . 48 }	. . . . . 45
	{ Tuberculosis . . . . . 2 }	

Vaccinated 6-12 months previously . . . . . 758

Alive	{ Contacts . . . . . 355 }	. . . . . 691
	{ Non-contacts . . . . . 386 }	
Dead	{ Non-tuberculous disease . . . . . 53 }	. . . . . 62
	{ Tuberculosis . . . . . 9 }	

Total number of survivors in contact with phthisical

subjects over a period of 6-18 months . . . . . 586

Deaths from all causes in traced series. . . . . 107

(8.1 per cent.)

Deaths from tuberculosis in traced series . . . . . 11

(0.8 per cent.)

Average French death-rate, 0-12 months, all causes

(Calmette, 1928) . . . . . 9.5 per cent.

The presentation of the figures by their author leaves much to be desired; since the majority of the infants were lost sight of, and amongst such as were followed the statistics do not give the incidence of tuberculosis amongst the vaccinated children who were exposed to familial infection as compared with its incidence amongst the vaccinated children not so exposed; nor is there any contemporaneous comparison made with children living under similar conditions who were not treated by B.C.G. Nevertheless, the results, when taken at their face value and contrasted with the previously quoted figures for children living with phthisical parents, appear somewhat striking.

At this period Calmette began to issue his vaccine to such medical practitioners who desired to make use of it, and in 1927 its utilisation in selected cases was recommended by the Ministère

du Travail, de l'Hygiène, de l'Assistance et de la Prévoyance Sociales. Calmette counselled its use only in the case of children directly exposed to tuberculous infection, but did not by any means impose this condition, nor does he appear to have respected it in his own work. He recommended the giving of three doses of 10 mg. of a recent culture of the bacilli at forty-eight hours' intervals, commencing as soon after birth as possible. He believes that the intestinal mucosa of the infant is temporarily and peculiarly apt for the penetration of bacteria, and states that the efficacy of the vaccine depends upon the "impregnation of the lymphatic organs" with numerous living bacilli of this type. He reports that in certain cases a slight generalised glandular enlargement occurs, 15-20 days after the vaccine has been administered, which, after lasting for two to three weeks, gradually disappears.

A further report of results obtained between 1924-27 was issued in 1928 by Calmette, in collaboration with Guérin, Bouquet and Nègre. In this period a total of over 50,000 infants had been submitted to the treatment with B.C.G. The results presented in this latest report are abstracted and analysed below.

- A. From July 1st, 1924, to December 1st, 1927, the number of infants treated = 52,772
- B. Of these 6,219 lived in contact with tuberculous subjects ;
- C. Of these 5,749 were followed up and their history is recorded ;
- D. Of these 3,808 had been vaccinated for less than a year at the time of reporting ;
- E. Of these 118 were dead (12.3 per cent. of series D) ;
- F. Of these 34 died of tuberculosis (*i.e.*, 0.9 per cent. of series D).

Since, as Calmette says, the death-rate in children living under such conditions is from 24 to 70 per cent. annually, the results achieved would seem striking.

One disadvantage of this method of presentation of results is that the actual duration of the experiment is unknown. "Less than a year" is a vague quantity, and if we assume that the average duration of life (*i.e.*, of the experiment) was six months, then the mortality figures must be doubled to enable anything like a comparison to be made with normal mortality figures. It is obviously misleading to compare the mortality in series observed

for "less than a year" with the annual death-rate in similar series.

In another part of the same paper other figures are given which are of great interest, although their number is small. They are included in the above-mentioned group of 6,219 infants who lived in contact with tuberculous subjects, but were separately followed up and reported upon by Dr. Ott, of Rouen. All of the cases in this small series were situated in the Seine Inférieure, a department in which tuberculosis is very prevalent. Although here also there is a lack of complete information, and a tendency to make rather unsound comparisons, we may abstract certain of the findings: 1,869 cases were treated with B.C.G. during a period of three years, of which ninety-three died below the age of one year, seven of them perishing from tuberculosis. Taking this portion of the series alone, for the complete figures are wanting, we find:—

*Amongst the Inoculated (Contacts). Age 0-1*

Total Deaths	Deaths from Tuberculosis	Percentage of all Deaths due to Tubercle
98	7	7.5

In the same department, in the year 1924, out of 2,071 infants dying below the age of one year, seventy-seven died from "tuberculosis," and ninety-two from "meningitis." Calmette assumes that in all cases the cause of the latter was tuberculosis. If we agree for the purpose of argument, we find:—

*Amongst the Uninoculated (Mainly Non-contacts) Age 0-1*

Total Deaths	Deaths from Tuberculosis	Percentage of all Deaths due to Tubercle
2,071	169	8.1

Similarly, analysing Calmette's larger series of inoculated contact cases we find :—

Total Deaths	Deaths from Tuberculosis	Percentage of all Deaths due to Tubercle.
118	84	28.8

The small series of Dr. Ott, which is the nearest approach we have been able to obtain to an exact analysis, shows within its limitations that the death rate from tuberculosis is, to put it at its very worst, at all events no higher in the treated cases than in the general population, whilst the larger series shows that in spite of the vaccine tuberculosis remains an important cause of death amongst the treated. In all fairness to the investigation, it must nevertheless be remembered that the series dealt with by Calmette and his collaborators are the very opposite of unselected ones, and it is quite possible, as is claimed, that the percentage of deaths due to tuberculosis, in untreated series in which contact with consumptives was close, would rise to very much higher figures than those given by the vaccinated cases.

At the same time, until the results are analysed with greater strictness and until we can learn in exactly similar series what the death rate from tuberculosis is amongst those who have had B.C.G. and in those who have not, we cannot properly evaluate the effects of the treatment. Such figures have not yet been made available.

The feeling of the writer is that whilst the results given are extremely suggestive and of sufficient importance to demand the most careful attention, immediate sweeping deductions from them are to be accepted only with the greatest reserve.

Calmette believes that the immunising effect of his vaccine is only slowly developed. From animal experiments, he thinks the latent period to be about twenty-five days, and he points out that

during this time the infant is not protected in any way. He considers that the days immediately succeeding birth are the period of election for the treatment, believing that the permeability of the alimentary mucosa becomes greatly lessened after about the fifteenth day of extrauterine life, and that consequently from this time onwards the absorption of the bacilli is effected with difficulty. In his earlier work he believed the immunity to be of short duration, but latterly he has come to consider that it may last for as long as five years. Calmette quite definitely believes that if this is so it represents a persistence of the living bacilli in the lymphatic system over this long period; although he admits that in the human subject, as time passes, the matter becomes complicated by the normally occurring process of the occurrence of small spontaneous infections with virulent bacilli. It is somewhat hard to conceive of these non-pathogenic organisms persisting alive in the tissues over periods of years, in the presence of phagocytes and antibodies which it is admitted that they call forth, and it is not clear why the distinguished author does not admit the possibility of an antituberculous immunity apart from the existence of live tubercle bacilli in the tissues. Nevertheless, Calmette states that he has found the bacilli in the glands of an infant six months after their ingestion, and they have also been found, apparently intact, in the tissues of calves more than a year after infection.

It may be recalled in this connection that in certain experiments Topley (1926) found *B. aertrycke* alive in the spleens of 88 per cent. of mice into which the organisms had been injected twenty-one days previously; if such long survival is possible in the case of an organism of this type it may well be believed that such a one as *B. tuberculosis*, which habitually resides for long periods in the tissues, may have a much more lengthy survival.

Infection with B.C.G., according to Calmette, does not necessarily result in the production of a positive tuberculin test, even though the bacilli are existing alive in the tissues. This point has been seized upon by German critics as indicating that no true antituberculous immunity is established. Calmette replies that the tuberculin test is only positive where active tuberculous

(follicular) lesions have developed ; which is normally not the case with the B.C.G. infection. This is not particularly convincing, but it seems certainly the case that the tuberculin test may be negative although there is an infection with the attenuated bacilli present : the matter awaits further investigation.

Since the view is taken that the absence of a positive tuberculin reaction is indicative of freedom from any tuberculous infection, the fear which Calmette has expressed that treatment with B.C.G. may set up dangerous Koch reactions does not apply to cases which present a persistently negative reaction. Theoretically, then, the treatment may be applied to persons of any age who are not excluded by this test. The chief scope for this would appear to be in native races in which tuberculization is not so common a process as in the more immune white races, and in which the mortality from the disease is usually very high. It has been put into practice by Calmette and his school in Indo-China, Madagascar, and Senegal, in the case of natives who are especially exposed to risks of tuberculous infection.

The procedure has also been used by Heimbeck (1928), in Norway, amongst certain hospital nurses in whom the risks of tuberculosis are high. Fifty-three per cent. of these upon entry on their course of training show a negative von Pirquet reaction, and the incidence of tuberculosis amongst such as reacted in this way was found to reach the amazing figure of 23 per cent., whilst amongst those reacting positively to the test only 0.9 per cent. contracted the disease. After experimenting upon himself with a subcutaneous injection of 0.2 mg. of B.C.G., which produced a small localised cold abscess, Heimbeck reduced the dose to 0.05 mg. of the culture, given subcutaneously, and proceeded to apply the treatment to others. The only immediate results of the injection were a localised infiltration, and in a few cases a small cold abscess. Having made these initial observations upon hospital nurses, this worker extended the treatment to other persons, and at the time of reporting had inoculated 522 subjects, all of which had previously reacted negatively to the von Pirquet test. In the majority of these the test became positive after the treatment. In the short period covered by his observations Heimbeck reports

that no cases of tuberculosis had occurred amongst the series of inoculated nurses.

**The Cultivation of B.C.G.**—Having achieved what he considered a satisfactory attenuation of the organism, Calmette has abandoned bile-containing media and continues the propagation of the bacillus upon ordinary laboratory media such as are used for the cultivation of the tubercle bacillus, especially upon glycerin-potato. For the production of large masses of growth, Santon's synthetic medium is recommended. Its composition is:—

Asparagine	.	.	.	.	.	4 gm.
Glycerin (pure)	.	.	.	.	.	60 „
Citric acid	.	.	.	.	.	2 „
Dipotassium phosphate	.	.	.	.	.	0.5 „
Magnesium phosphate	.	.	.	.	.	0.5 „
Iron ammonium citrate	.	.	.	.	.	0.05 „
Water	.	.	.	.	.	940 c c.

Adjust to  $pH = 7.2$ , with ammonia, and sterilise by autoclaving. It is interesting to note that in this medium the B.C.G. produces an active tuberculin.

Guérin (1927) states that the cultures used for immunisation should not be more than twenty-five days old, and that the emulsion which is prepared from them for human absorption should be used within ten days of its preparation, otherwise the bacilli rapidly die out. He finds, however, that by preserving cultures in the cold ( $-7^{\circ} C.$ ) their activity is but little diminished after two months.

The work and views set forth in the preceding pages have aroused widespread interest, and a good deal has been done and written upon the matter since it was first brought to notice. Tscekhnovitzer, on behalf of a Commission of the Ukraine, has reported extensive experiments upon animals, including 224 guinea-pigs and 112 rabbits, in an investigation of the pathogenicity of B.C.G. as a preliminary to its use in the human subject. None of the guinea-pigs infected developed active tuberculosis, although, as a result of the intraperitoneal and subcutaneous

injection of large doses, cold abscesses formed containing acid-fast bacilli. The contents of these abscesses, injected into fresh animals, failed to cause tuberculosis. It was also found that following on intravenous injections, and other observers have noted the same effect, more especially in the lungs of rabbits, that numerous small, greyish lesions developed, having a naked-eye appearance exactly resembling the smallest miliary tubercles. These however became absorbed, and in animals killed six months later they had disappeared. Calmette and his associates fully admit this occurrence, when large intravenous doses of the bacilli are given, but they state, and here Tscekhnovitzer agrees with them, that the lesions are avirulent for guinea-pigs, and upon attempts at passage the organisms disappear.

It has been the finding of several pathologists who have especially investigated this aspect of the question, that the claims made by Calmette for the protective effect of B.C.G. vaccination in laboratory animals have been exaggerated. Heymans (1926) found that vaccinated rabbits and guinea-pigs all succumbed eventually, when infected with a virulent strain of bovine tubercle bacilli, but that their period of survival was approximately double that of the unvaccinated controls. At the same time he failed to find any protective effect of the vaccine upon animals exposed to infection by cohabitation. It may be noted that he used, as his test strain, a culture of bovine origin of a virulence which had been exalted by passage until it had become constant. Somewhat similar results were obtained by Dwijkoff and Masourowski, who tested their vaccinated animals both with virulent and attenuated strains of tubercle bacilli. In the first case the animals succumbed to the infection, but this fatal termination was delayed in the vaccinated animals and, on histological examination, an increased tendency to fibrosis was seen in the lesions, more especially in those of the liver and lymphatic glands. With a strain of bacilli "*peu virulente*," the animals resisted the infection, but no information is vouchsafed in these workers' papers as to the effect of this strain upon control animals.

Some careful experiments to test the value of B.C.G. in protecting guinea-pigs were carried out by Okell and Parish (1928).

They found, as has been noted above, that the general effect of such vaccination (they used only subcutaneous or intravenous injection) was merely to prolong the life of the animals and that it did not prevent infection in the case of a virulent test organism. When, however, they adjusted the conditions of their experiments with great care, and infected the treated animals by the conjunctival route with a minimum infecting dose, they found that out of twenty-two vaccinated guinea-pigs six remained free from infection, whilst of thirty-two control animals all succumbed. They further found that the vaccine was completely devoid of pathogenicity for the animals, even five doses of 20 mg. failing to affect them.

It is clearly evident from the work which has been quoted that the vaccine itself is non-pathogenic, and it further appears that in carefully adjusted experiments it will afford, in certain cases, a complete protection even to so sensitive an animal as the guinea-pig. Since in other animals, and amongst them man, we have reason to believe that the natural resistance to tuberculosis is of a considerably higher order, it is reasonable to suppose that in these complete protection should be much more easily accomplished.

Some experiments, bearing rather directly upon the efficacy of vaccination by the ingestion of B.C.G., upon which those who do not share Calmette's views of the prime importance of the alimentary route of infection will have serious doubts, have been carried out on guinea-pigs by Satake (1927). This worker injected the vaccinating bacilli, in either 1.0 or 5.0 mg. doses, directly into the stomach through the abdominal wall, by means of a fine needle. A month later all the animals received a milligramme of a virulent culture of human tubercle bacilli by the same route. Satake states that in control experiments he established the fact that this injection of virulent bacilli was not followed by any local lesion at the seat of inoculation nor by tuberculosis of the peritoneum; but that its regular result was the progressive enlargement and tuberculosis of the spleen and abdominal glands, more especially of the duodenal glands. The change was well marked in the second week following the injection, and the last-named glands had attained the size of a bean at the end of the third week.

In the case of the animals previously receiving B.C.G., these glands showed little or no enlargement three weeks after the injection of virulent bacilli. On histological examination they showed some general hyperplastic and hyperæmic changes, with evidence of increased activity, but no recognisable tuberculous lesions were found. These experiments go to show that ingestion can produce the same effects as have been reported for the injection of B.C.G., viz., a slowing down of the rate of infection; but since they were not pursued beyond the third week no information is available as to the late results or the ultimate fate of the infected animals.

Calmette's claims have been criticised from another point of view. It has been urged that the organisms in his vaccine are not so completely deprived of virulence as their author claims to be the case, and that there is a considerable danger of this quality being recovered in the animal body (Medin, Kraus, von Pirquet, Lowenstein). The majority of workers who have actually tested the qualities of the B.C.G. culture have testified to its complete innocuousness, and such attempts as have been made to restore its virulence by passage have failed (Tscekhnovitzer, 1928). Although theoretically it should be possible to do this by continued endeavour towards this end, there is at present no evidence of the spontaneous recovery of virulence in the tissues.

In respect of Calmette's statements that the modified organisms never provoke true tuberculous lesions and that the tubercle-like lesions seen in heavily-infected animals, which end by being absorbed, are "lymphoid" reactions and essentially different, it is interesting to note some experiments carried out by Maximow (1928) upon the effects of B.C.G. and virulent tubercle bacilli on tissue cultures. Maximow points out that the effect of ordinary bovine bacilli, upon a mixed tissue culture from a lymphatic gland of the rabbit, is an early and complete destruction of the epithelioid class of cell, which undergoes degenerative changes even though not in close contact with the bacilli. Fibroblasts are more resistant, but they too, in the end, undergo degeneration. In the case of similar cultures contaminated with B.C.G. no evidence is seen of any noxious action of the organisms upon the cells. The epithe-

loid cells grow around the bacilli, ingest them and seem to live in comfortable symbiosis with them, whilst the fibroblasts pursue their normal growth. If the bacilli are few in number they are destroyed by the proliferative cells. In no case is any evidence of toxic action upon the cells forthcoming, and the bacteria can be observed multiplying within the cells' protoplasm without the latter appearing to suffer from their presence.

A criticism, more weighty than philosophical speculations upon possible changes in virulence, has been put forward by Petroff, Branch and Steenken (1922). These workers claim that on growing a strain of B.C.G., provided by Calmette, on medium containing gentian violet, two types of colony were produced. This proved to be the familiar process of microbic dissociation (p. 39), and eventually S and R varieties were obtained. Of these the R colony behaved in the way expected of B.C.G., but the S colony proved virulent and produced generalised tuberculosis on inoculation into guinea-pigs. In the same paper a passage experiment is recorded, in which virulent tubercle bacilli emerged in the inoculated animals; these in culture resembled the S strain. This finding is one of importance, and its early further investigation a matter of urgency in view of the spreading use of B.C.G. That the modification produced by Calmette may ultimately be found to square with the facts of bacterial variation, now being so widely demonstrated, is only likely, but it is a disturbing thought that virulent variants may reappear with the ease with which they seem to have done in the hands of Petroff and his colleagues; who further state that they obtained similar results with another strain of the organism. In general, the variant types (R), which are non-virulent, are pretty permanent upon ordinary laboratory media, and though reversions have been recorded the general trend of opinion is that these do not occur at all readily. In the case of Pasteur's modified anthrax vaccines, the loss of virulence has been a character of remarkable fixity. It may be that the particular medium employed constitutes the condition necessary for such a reversion, to which there may be no tendency on the more usual laboratory media; it being well known that dyes and inhibitory substances in general are active agents in promoting

such changes, although their recorded effects have generally been in the opposite direction.

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## CHAPTER VII

### ULTRAMICROSCOPIC AND FILTER-PASSING VIRUSES

THE diameter of the staphylococcus is just under  $1\mu$ . That of the smallest particle visible under a good oil-immersion lens lies between  $0.1$  and  $0.2\mu$ .<sup>\*</sup> The true size and form of any object whose dimensions are less than a half of the wave-length of the light used to illuminate it cannot be correctly observed on account of the diffraction which takes place. The wave-lengths of the rays of the visible spectrum lie between  $400$  and  $700\mu\mu$ . The invisible ultraviolet rays, to which the photographic plate is sensitive, are much shorter. Kohler (1904) showed the practicability of utilising this fact in microphotography, and Barnard (1925) has devised a photomicrographic apparatus by which it is possible to photograph objects thus illuminated, whereby he believes that he has recorded the appearance of the virus of malignant disease described by Gye.

Beyond the range of visible objects are particles of a smaller size, designated submicrons, which are visible by the ultramicroscope and which have a diameter of between  $0.1\mu$  and  $1.0\mu\mu$ . They constitute the colloidal solutions. Beyond these come the yet smaller molecules of true solutions which, in the phraseology of colloidal chemistry, are termed amicrons.

Our purview of bacteriology is, then, very generally limited by the oil-immersion lens, for although the ultramicroscope permits us to recognise particles as small as  $0.005\mu$ , it only shows diffraction images, all small particles appearing indifferently as illuminated granules and no information being conveyed as to their structure or form. Even with this method an absence of visible particles does not necessarily mean that those present are so small as to be incapable of illumination; it may mean that their refractive index

<sup>\*</sup>  $1,000\mu\mu = 1\mu = 0.001\text{ mm}$

is not sufficiently different from that of the dispersion medium for diffraction to take place.

The possible lower limit of living particles is not certainly known, but it may safely be assumed that viable particles of less than  $0.1\mu$  may exist, since certain micrococci are known whose dimensions are about those of the lower limit of visible particles. Frei has calculated, from a consideration of the size of the molecules necessary to give the required complexity, that the size of the smallest living body possible may be between 2 to  $5\mu\mu$ . From the table which follows it would appear that this limit has been set too low. Certain of the estimated dimensions of small bacteria and colloidal particles may be quoted here as affording some information for comparative purposes.

#### A COMPARISON OF THE SIZES OF CERTAIN PARTICLES AS GIVEN BY VARIOUS AUTHORS

Submicrons—particles between  $0.15\mu$  and  $0.005\mu$ .

Amicrons—particles below  $5\mu\mu$ .

H. molecule—calculated at about  $0.16\mu\mu$ .

Saccharose molecule—calculated at about  $0.7\mu\mu$  (Ostwald).

Starch molecule—calculated at about  $5\mu\mu$  (de Bruyn and Wolff).

Albumin molecule—calculated at about  $4-10\mu\mu$  (Bechhold).

Gold sols—calculated at from  $6-95\mu\mu$  (Zsigmondy).

Bacteriophage corpuscles—calculated at about  $20-30\mu\mu$  (Prausnitz, and von Angerer).

Mouse sarcoma virus—calculated at about  $75\mu\mu$  (Barnard).

*B. pneumosintes*— $150-300\mu\mu$  ( $0.15-0.3\mu$ , Olitsky and Gates).

Diameter of *L. icterohæmorrhagiæ*— $200\mu\mu$  ( $0.2\mu$ ).

Strongyloplasmata— $150-200\mu\mu$  ( $0.15-0.2\mu$ , Lipschutz).

*Rickettsia prowazeki*— $200-300\mu\mu$  ( $0.2-0.3\mu$ , Sergent).

*M. prodigiosus*— $500\mu\mu$  ( $0.5\mu$ ).

The first demonstration that disease may be caused by materials which are non-particulate to ordinary bacteriological analysis, but which possess some of the attributes of living organisms, was made by Löffler and Frosch in 1893, who showed that the filtered,

bacterial-free, vesicular lymph from an animal suffering from foot-and-mouth disease was capable of conveying the infection to a normal animal, and since this could be transmitted from animal to animal in series, they concluded that they were dealing with a reproducing micro-organism and not with an inanimate virus. From these observations have sprung the investigation of what are variously referred to as "ultra-microscopic organisms," "filter-passing organisms," and the organisms causal of "virus diseases."

It has been widely assumed that these active agents are living micro-organisms; smaller beings, but on much the same plan as the bacteria with which we are familiar; but some considerations, notably the remarkable phenomena disclosed by d'Herelle, make this no longer certain.

It is a useful exercise to consider what would be the position of the bacteriologist if deprived of his higher-powered objectives. He would note that certain products obtained from disease and injected into animals would give rise to like results in these animals, and he would frequently find the diseases so produced capable of transmission in series. He might notice certain changes in culture media, and therefrom assume the presence of lowly forms of life, of which he would not be able to see the units individually and for which he would probably look for analogy to the larger protozoa. He might remark that these invisible organisms had a tendency to form masses, a feature which was absent from many of his familiar parasites, such as amœbæ and trypanosomes. Most of his knowledge, however, would be inferential and coloured, to a degree which we can hardly gauge, by ideas engrained by the study of larger forms. None of it would be direct.

The position with regard to the ultramicroscopic "organisms" is somewhat similar. We can observe the effects of their presence, we can study certain of their properties in ways we have indicated, but we always have to turn again to the demonstration of their specific effects for evidence of their existence in any materials. Thus the bacteriophage can only be demonstrated by the production of bacterial lysis, and the virus of foot-and-mouth disease by the production of vesicles. Our conceptions of these infecting agents are largely dominated by those of ordinary bacteriology.

Attempts at cultivation are unsatisfactory. Of the existence of saprophytic forms, akin to the pathogenic ones, we know practically nothing, and therefore many of our supposedly pure forms may be contaminated. In fact, it is harder to conceive of a problem with which in the present state of our knowledge we are less fitted to grapple. Not until some very considerable advance has been made, possibly of a technical nature, for which the time is now ripe, may we expect much light upon the problem of these obscure agents. When once the next essential step has been taken we may look forward to rapid advances and a new and very fertile period in bacteriology.

Most of the current conceptions of the ultramicroscopic viruses regard them as particulate bodies in the ordinary bacteriological sense. It seems very doubtful if this is the case at all, and Beijerinck's old and scorned conception of a *contagium vivum fluidum* may be much nearer the mark than is usually admitted. It seems to the writer that of recent discoveries which bear most directly upon the solution of the problem, that of d'Herelle may well prove to be the most important.

Probably the most widely used criteria of the ultramicroscopic viruses is their filterability. Ordinary bacterial filters, either of the earthenware type, of which the Pasteur-Chamberland is the best known, or the diatomaceous earth filter of Berkefeld, will prevent the passage of bacteria when these are present in the filtered fluid, but do not prevent the passage of the agents of the diseases we are now studying. A very large number of infections have been from time to time described as due to filtrable viruses. The following list is by no means exhaustive.—

Poliomyelitis	*Typhus
Dengue	*Herpes zoster
*Variola	*Common cold
*Vaccinia	*Trench fever
Molluscum contagiosum	Hog cholera
Trachoma	Foot-and-mouth disease
Mumps	Rinderpest
Rabies	Fowl pox
Herpes febrilis	Fowl plague

*Varicella	Infectious anæmia of horses
*Measles	African horse sickness
*Influenza	Bovine pleuro-pneumonia
*Pellagra	Rous sarcoma of fowls
*Epidemic encephalitis	Mosaic disease of plants
Australian " X disease "	Canine distemper.
Yellow fever	

[Some of these maladies, marked with an asterisk, are for one reason or another suspect, but in the others the infective element is generally agreed to be filtrable.]

In this list is included bovine pleuro-pneumonia, which is in a much more satisfactory position than most of the other conditions, as the virus has been cultivated by Nocard and Roux, and since then by many others. It is also just within the limits of microscopic visibility and only passes the coarser filters

The criterion of filtrability is an extremely loosely used one, and under the best circumstances unsatisfactory. A filter is a mass of holes and irregular passages, and whether or not a living particulate virus will pass depends upon the relationship of the largest of these crevices to the smallest viable particle of organismal protoplasm, and to chance favouring the passage of the one by the other, a set of conditions rarely the same in different experiments.

A great number of factors influence the act of filtration. The viscosity of the filtered material; the temperature at which filtration is carried out; the pressure which is maintained during the process, the presence and concentration of electrolytes, the concentration of the solution, the time occupied by filtration; and the type of filter used.

When such experiments are made these conditions should be standardised as much as is possible. Loose reports that such and such a virus "passes a Berkefeld filter" are often worse than valueless since they may be misleading. Of the various types of filters available the Berkefeld V. is probably the most widely used and the worst; it is extremely porous, very fragile, and only cleaned with difficulty. In some experiments, performed by the

writer, it was found that using a high vacuum three Berkefeld V. filters out of four allowed Pfeiffer's bacillus to pass.

The Berkefeld filter is manufactured in three grades marked, according to their permeability for water, as follows :

V. (viel) : the coarsest grade.

N. (normal) : the intermediate grade.

W. (wenig) : the finest grade.

A much better filter is the Pasteur-Chamberland, of which a laboratory type is put on the market in the form of jointless candles in nine grades. The numbers and relationship of these to the larger and better known candles of this type are shown below.

Laboratory types :—L-1 ; L-1a ; L-2 ; L-3 ; L-5 ; L-7 ; L-9 ; L-11 ; L-13.

Ordinary large P-C filter candles :—

||       ||  
" F "   " B "

The makers, and others, state that :

L-1 is for clarifying purposes only, not for keeping back organisms.

L-1a { arrest the large micro-organisms, but pass the smaller,  
L-2 { such as bovine pleuro-pneumonia. Pass typhus and  
      influenza viruses (Nicolle and Lebailly).

L-3 arrests tetanus spores. Passes foot-and-mouth virus (Bedson).

L-5 Particles of  $0.2\mu$  said to pass (Rosenthal). Passes the viruses of bovine pleuro-pneumonia ; foot-and-mouth disease (Remlinger), fowl plague (Dorset), trench fever (?) (Am. Red Cross Commn.), yellow fever (Marchoux and Simond). Arrests the passage of rabies virus (Remlinger). allows this to pass (Poor and Steinhardt).

L-7 Keeps back the viruses of foot-and-mouth disease (Remlinger), pleuropneumonia (Dorset); yellow fever (Marchoux and Simond). Passes that of hog cholera (Dorset).

L-9

L-11

L-13 passes the bacteriophage (d'Hérelle)

There is, of course, no assurance that any two filters nominally of the same grade will be identical in practice, since in such structures a great deal of variation is bound to occur even when the conditions of manufacture are most carefully standardised. An estimate of the rate at which distilled water will filter through under a given pressure is a rough general method of comparing filters and of testing for leakage.

Most infected fluids are highly albuminous and require to be well diluted, and rendered as free as possible from gross particles, before filtration is attempted. Albuminous material tends to be adsorbed upon the filter, as the well-known experiments with toxin and complement show, and it must not be forgotten that the finer the grade of filter the greater will be the amount of adsorption going on, and consequently the greater the loss of active substance from this factor. Thus, with a not very active preparation, complete inactivity after filtration may be traceable entirely to this and have little to do with the size of any active particles and those of the filter's pores. The charge upon the particles may also result in their being precipitated in the pores of the filter, should this be charged oppositely; so that from several causes it becomes progressively less permeable as the operation proceeds. The control which is sometimes applied, of testing the impermeability of a filter for *B prodigiosus* at the end of an experiment, is of course useless, except in a positive sense, on account of the clogging of the filter's pores which occurs during the operation.

It may also be said that similar criticisms to those directed against filtration hold for various other methods of examining the properties of viruses, such as the effect of centrifugalisation, when these are present in pathological products such as infected brain, tissue pulp, etc. Here, too, the virulent agent is bound to be very largely adsorbed upon the non-specific particles and will be distributed with them. The force of this is shown by some experiments of Levaditi and Nicolau (1923), who showed that rabies, vaccinia, and encephalitis (herpes) viruses were capable of being wholly adsorbed from solutions by animal charcoal and other substances, as well as upon the precipitated globulin of protein solutions, in this way behaving like toxins.

C. J. Martin, in 1896, introduced the procedure of ultra-filtration, using filter bougies impregnated with gelatin, and the method has been adapted by Bechhold (1919) to the problems of colloidal chemistry. With improving technique, and the now realised possibility of preparing filters of variable porosity and of fairly stout structure, this method may throw some further light upon the relative size of the active particles present, although still open to most of the drawbacks of filtration in general.

Levaditi (1928) found that certain viruses which are capable of producing cerebral lesions would traverse collodion membranes under a pressure of 10 to 12 cm. of mercury. The sacs used, which freely allowed passage of the bacteriophage but which "more or less completely" held back such substances as complement, hæmolytic antibody, trypsin, and diphtheria and tetanus toxins, were permeated by the rabies virus once in five experiments, by the encephalitis (herpetic) virus thrice in eight experiments; and by the vaccinia virus seven times in eight experiments. Levaditi concludes that these ultramicroscopic viruses are protein aggregates which approximate in size to those which constitute the unorganised ferments and microbial toxins. Bedson (1927), on the other hand, in carefully controlled experiments, was totally unable to filter the herpetic virus even through 1 per cent collodion membranes, and suggests that positive results have been due to leaks. It is evident that the whole question needs re-examination with a standardised technique.

The properties of the infective agents of the ultramicroscopic type vary considerably, and though attempts have been made to classify them according to characters such as the site of their activities, resistance to glycerination, their association with inclusion bodies (chlamydozoa or strongyloplasmata), etc., such groupings have little meaning at the present stage of our knowledge.

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### ACUTE POLIOMYELITIS

This is one of the diseases of this group upon which a very great deal of attention has been focussed and to which some of the ablest experimenters have given much study. The work done upon it has also served as a model for many later investigations, so that we may well consider it as an introduction to the subject.

The important findings in connection with this disease, from the bacteriological point of view, resolve themselves into —

- (1) The successful transmission of the disease to animals,
- (2) The study of the properties of the virus;
- (3) The cultivation of the virus.

The transmission of the disease to *cynocephalus* and *macacus* monkeys was achieved in 1909 by Landsteiner and Popper, who injected an emulsion of the spinal cord of a child, which had died in the fourth day of the disease, into the peritoneal cavities of two of these animals. Both monkeys became infected, one becoming ill on the sixth day and dying on the eighteenth, the other becoming paralysed in the lower limbs seventeen days after the injection and being killed two days later. The histological appearances in these animals were similar to those seen in the spontaneous disease in man. Cultural experiments with the infective material were negative, as were also attempts to transmit the disease to guinea-pigs, rabbits and mice. Landsteiner and Popper did not succeed in an effort to transmit the disease from monkey to monkey.

Following quickly upon these results came confirmation by other workers, including Leiner and Wiesner in Vienna, Landsteiner and Levaditi in Paris, and Flexner and Lewis in America, all of whom succeeded in transmitting the disease serially from animal to animal, thus rendering its further experimental study possible.

In 1918 McIntosh and Turnbull, in this country, obtained similar

results, thus confirming the similarity of the virus of English cases to that present on the Continent and in America. All these successful results were achieved by the intracerebral inoculation of emulsified spinal cord from fatal cases, which subsequent experiments showed to be the procedure of choice for the transmission of the malady.

The virus of poliomyelitis has no affinity for other of the lower animals, with the possible exception of the rabbit. Experiments with this animal have given rather conflicting results. Whilst a majority of workers find the animal to be insusceptible, it is claimed by others that whereas all rabbits are not susceptible to the virus, certain of them may be so, and, further, that individual strains of virus may be more pathogenic for rabbits than others are. The disease, as described in the rabbit, does not resemble human or simian poliomyelitis, but takes the form of convulsive seizures, rapidly leading to death (Marks, 1911). The positive results which have been obtained are not associated with any of the typical histological appearances of the human disease, but have nevertheless been claimed to be specific, since the central nervous system of the diseased rabbits will induce typical poliomyelitis when inoculated into apes. This is not, however, entirely conclusive, since it has been shown that the poliomyelitic virus can survive for considerable periods in the tissues of the rabbit.

**The General Properties of the Virus.**—Over 90 per cent. of monkeys appear to be susceptible to inoculation with the virus. The clinical results and histological findings in these animals very closely recall those met with in man. The experiments upon the properties of the virus have for the most part been carried out with the emulsified cord material of monkeys.

Most of the workers upon this disease are agreed as to the filtrability of the organism, which will pass Berkefeld V. and certain Chamberland filters (grade unstated). There is some loss of activity by this process, since the incubation period with filtered materials is longer than is the case when the crude emulsion is used. The poliomyelitic virus belongs to the class which resist glycerination over long periods, and therefore has certain affinities

with that of rabies Flexner and Amos (1917) showed that on one occasion a portion of cord, preserved in 50 per cent. glycerin, was still active after a lapse of six years.

Different strains of the virus show differences in their pathogenic effects. Some regularly lead to death, whilst others appear to be insufficiently pathogenic to infect the experimental animal. Flexner (1924) has shown that a strain of the virus, of high initial pathogenicity, may undergo marked fluctuations in virulence during its preservation over a period of years, for which no explanation has been forthcoming. He also isolated a strain of poliomyelitic virus which infected monkeys with regularity, but produced only a mild form of the disease which was recovered from. Once recovery had taken place, the animals were immune to reinoculation with a virulent strain. Zinsser (1912) states that Landry's paralysis is merely a form of acute poliomyelitis, and that he has obtained typical results in monkeys by their inoculation with human material from a case of this disease. This view is also held by Levaditi.

The infection can be conveyed to animals by inoculation by the subcutaneous, intraperitoneal, intravenous, intraocular and intracranial routes. The last named is the most certain method of producing the experimental infection, whilst the blood route is not very favourable to the development of the disease. Flexner and Amos (1917) have shown that in health there is no great tendency for the virus to pass from the blood stream into the nervous substance, but that by inducing an aseptic inflammation in the meninges this passage is facilitated. The route of infection which appears of most importance in the spontaneous disease is by way of the respiratory passages, and the experiments of Leiner and Wiesner, Landsteiner and Levaditi, and of Flexner and Lewis, have shown that the nasopharynx harbours the virus in disease, and offers a means of ingress. It would appear probable that the intact and healthy mucosa is not readily penetrated by the virus, as Levaditi has found experimentally, but that upon the supervention of trauma, or an inflammatory alteration, the virus effects a passage. Once having gained the submucosal tissue, the infection may spread along the filaments of the first

nerve to reach the olfactory lobes, medulla and cord. In addition to this, the tonsils, pharyngeal mucosa, salivary glands and intestinal contents have been shown, at different times, to harbour the virus, which it would appear can reach the central nervous system by the lymphatic paths as well as by travelling inwards, in common with the virus of rabies and tetanus toxin, along the peripheral nerve trunks. Both Flexner and Lewis, and Levaditi and Landsteiner, have shown that inoculation into the peripheral nerves will occasion the disease, and that under such circumstances the groups of muscles first affected are those of the area supplied by the particular nerve used for the inoculation.

With regard to the question of immunity, it has been established by all observers that an animal having recovered from the disease is refractory. At the same time antibodies are developed in the body fluids, since the serum of experimental animals which have recovered from the disease, and that of human convalescents, have a neutralising effect upon the virus, *in vitro*. Attempts have been made to extend this property to the treatment of the disease, but with very questionable success. It would seem that here, as with certain other nervous affections, once the virus has commenced its destructive effect upon the nerve-cell, which is a matter very speedily accomplished, the administration of antibodies has no appreciable curative effect.

Many workers have attempted to render monkeys refractory to the disease by the inoculation of the cord virus, attenuated by drying, dilution, heating, and treatment with immune serum or with chemicals, all of which methods have given successful results; so that an active immunity is possible in this disease as in rabies, with which it has so many analogies. The immune state is, however, not at all easy or sure of production, and a certain number of animals contract the disease during the process of immunisation. Some observations of Flexner and Amos (1924) tend to suggest that the repeated injection of virus, in non-effective doses, may on occasions produce actual susceptibility rather than immunity. The method has no practical application in human medicine.

**The Cultivation of the Virus.**—Flexner and Noguchi claimed to

have done this in 1913, by means of the technique successfully used for the cultivation of the *Treponema pallidum*

Noguchi originally accomplished this in 1910-11 by cultivating the organism obtained from syphilitic orchitis in rabbits, in which it is present in enormous numbers, in high, narrow, tubes of serum-water, to which a small piece of fresh, sterile, kidney tissue had been added. The necessary conditions were the presence of the fresh tissue, strict anaerobiosis, a slightly alkaline reaction, and a temperature of 35° to 37° C. The growths in the first instance were contaminated, but were purified by allowing the organisms to grow through a Berkefeld filter, in which process the *S. pallida* outstripped the bacteria. Later on it was found that as the strains became accustomed to artificial cultivation, they would grow well in media of the above type, which were stiffened by a minimum (1.3 per cent) of agar. From a stab culture in such a medium the spirochaetes grow out from the needle track into the surrounding medium, and can be obtained in a pure state by sub-culture from the periphery. Noguchi only exceptionally succeeded in obtaining primary cultures, but found that when once growth was established, sub-cultures could be obtained with regularity. These sub-cultures were pathogenic for rabbits, producing a specific orchitis in a certain percentage of injected animals.

These are the methods which were applied by Flexner and Noguchi to the cultivation of the virus of poliomyelitis. Fresh pieces of infected monkey's brain and cord were placed in deep tubes of the media, which contained fresh, unheated, human aseptic fluid and sterile tissue. The tubes were covered with a layer of liquid paraffin, strict anaerobiosis not being necessary. After about five days' incubation an opalescence began to appear about the portion of tissue at the bottom of the tube, gradually extending upwards. The turbidity was due to the presence of minute rounded bodies, occurring in masses and short chains, which stained red by Giemsa's method. Primary cultures were obtained with difficulty, but secondary growths took place more readily, and could be obtained in the semi-solid media used for the cultivation of *T. pallidum*. Growths were also obtained from Berkefeld filtrates and from glycerinated brain. Flexner and Noguchi

believed that these "globoid bodies" represented the causal agent of poliomyelitis. They adduced, as further arguments in support of this, the facts that the organisms, or indistinguishable formations, could be found in the central nervous system of man and animals succumbing to the disease; that sub-cultures in the third generation would produce the typical disease in monkeys, which was associated with histological findings characteristic of poliomyelitis and in which the animal's nervous system was fully virulent for fresh animals; and, finally, upon the fact that the infectivity of nervous tissues for animals ran closely parallel to the degree of ease with which they yielded cultures of the globoid bodies. It is to be noted that in most cases their cultures rapidly became non-infective, although yielding heavy growths, and that only exceptionally did sub-cultures prove virulent for monkeys. This loss of virulence they regarded as an effect of artificial cultivation.

The method of Flexner and Noguchi is one of delicacy and abounds in uncertainties, and the results obtained have never been confirmed by workers outside of the Rockefeller Institute (Amos, 1917; Smilie, 1918). Even in the hands of its exponents the technique is full of difficulties, and such minor details as variations between different samples of ascitic fluid are said to make all the difference between success and failure. There are other unknown, and therefore uncontrollable, factors which may intervene, but Flexner states that increased confidence and competence come with familiarity with the technique. Once cultures are obtained, their continuation is a much easier matter, but in most cases they speedily become non-infective. Flexner, Noguchi and Amos (1915), however, claim to have succeeded in producing the disease in *macacus* monkeys with a strain of virus which had been in culture for eighteen months, and in this period had passed through a sufficient number of transfers to enable these authors to state the dilution of the original material approached infinity, they stated that the quantity of infective material was very greatly less than the smallest possible active dose of fresh brain emulsion. Once the organisms re-entered the animal body, and produced infection, their isolation from the nervous tissue was again as difficult as

ever, so that the adaptation to a saprophytic mode of growth seemed lost with the re-assumption of a parasitic existence. It may be noted that in these experiments it was found that untouched cultures remained alive for a matter of thirteen months, both at room temperature and at 37° C., so that the difficulties in cultivation of the organism were not due to any lack of viability

General opinion at the present day is against the acceptance of the work of Flexner and Noguchi, at any rate in its entirety. The methods employed have been widely applied all over the world to the cultivation of organisms in conditions of doubtful causation and in the virus diseases. In many of these, as will be recounted in the following pages, positive findings have been recorded, but none of them have stood the test of time or repetition, save in the case of the spirochætal diseases. In certain instances bodies akin to the globoid bodies have been observed, but rigid controls have robbed them of their promised significance. Later experience has made it certain that the Noguchi method is beset with fallacies, and that complex changes occur in the media which lead to the precipitation of non-specific protein particles which give an appearance of clouding, especially about the piece of tissue, and which falsely suggest growth.

Even if we dismiss the globoid bodies, this does not necessarily invalidate all of the conclusions arrived at by the Rockefeller workers as a result of the animal infections produced by their cultures. The question remains whether any pullulation of the virus in invisible form took place in the media employed. Here again doubts are widely felt, for in most of the reported cases the virus was only carried over a few generations. The inoculations from tube to tube were in most cases heavy, and in the first instance a mass of infective tissue was introduced into the culture. It is now known that certain viruses can diffuse into fluids in which they are surrounded (*e.g.*, herpes, rabies and the Rous sarcoma) in a way which was not fully understood at the time when Flexner and Noguchi's work was done, nor was it then realised how greatly the infecting agent may be diluted in some of these conditions and yet retain its activity.

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### THE ENCEPHALITIS-HERPES PROBLEM

Epidemic encephalitis (encephalitis lethargica) entered upon its modern history when it appeared in Central Europe in the third year of the war, and was reported from Vienna by von Economo in 1917. It reached this country late that year, and was observed clinically by W. Harris, who thought it was botulism, and by Arthur Hall, whose judgment was more reserved, in epidemic form in April, 1918. The disease reached America late in 1918, and spread across the North American continent from east to west. There is little doubt that the condition had existed prior to this modern epidemic, as an indistinguishable form of encephalitis is described by various clinical writers, notably by Dieulafoy, and the author well recollects a case of the disease which he saw when a house physician in 1912.

The malady was at first widely looked upon as an unusual form of Heine-Medin disease, and there was nothing in its clinical symptomatology to absolutely negative this possibility, although the very marked differences in age incidence, localisation, progress and termination soon made it clear that the disease was in all probability a type *sui generis*. It was left for the pathological anatomist and experimental investigator to supply the proof of this independence.

The experimental investigation of the disease has had an extraordinarily chequered career, being hedged about by contradictions, complications and false scents, the exact bearing of some of which have not yet been fully settled. The disease, which was made notifiable in England and Wales in December, 1918, has shown the following incidence to date —

CASES NOTIFIED ANNUALLY					ENGLAND AND WALES			
1919	1920	1921	1922	1923	1924	1925	1926	1927
541	889	1,470	451	1,028	5,086	2,634	2,267	1,615

After the high-water mark of 1924 the disease has progressively waned, and this, together with the fact that such cases as occur in this country are now largely gathered into the fever hospitals, has handicapped the further investigation of many of its outstanding problems

The apparent similarity of encephalitis to poliomyelitis immediately suggested the possibility of investigating it along similar lines. McIntosh, working for the Ministry of Health, took up this line of research, and in October, 1918, reported that he had investigated eight fatal cases of the disease by inoculating *Macacus rhesus* monkeys with the brain and cord tissue which had been preserved in 50 per cent. glycerin from three to four days. In no case did any definite paralysis develop, even after a prolonged period of observation. McIntosh, who had had previous experience of this type of work, having produced poliomyelitis in monkeys with virus from English sources in 1913, noted that up to the time of his report there was no record in the literature of the successful transmission of encephalitis lethargica to monkeys and concluded that the two diseases were essentially dissimilar.

Early in 1919 reports of an entirely different nature began to arrive from Strauss, Hirschfeld and Loewe at the Mount Sinai Hospital in New York. These workers claimed to have transferred the disease to monkeys, utilising emulsified brain tissue from a human case and also naso-pharyngeal washings as the sources of their virus. They further stated, in a later paper, that the rabbit was a suitable animal for such transmission experiments, and that the intracerebral inoculation of animals with cerebro-spinal fluid from human cases might yield positive results. The disease could be transmitted from rabbit to rabbit; was due to a virus in the central nervous system which would pass Berkefeld filters; and finally, upon cultivation by the Noguchi method, yielded a growth of minute bodies similar in appearance to those described by Flexner and Noguchi in poliomyelitis and capable on inoculation of producing all the results given by the infective brain. Thus the whole series of phenomena elucidated by the workers at poliomyelitis appeared to have been duplicated, but the two diseases were nevertheless considered distinct on account of the

preference of the virus of poliomyelitis for the monkey and of that of encephalitis for the rabbit

These results then received some support from McIntosh and Turnbull, who, late in 1919, reported that they had conveyed the disease to a Patas monkey by the simultaneous intracerebral and intraperitoneal inoculation of the Berkefeld-filtered emulsion of brain substance from a fatal case of encephalitis, the brain having been preserved for a month in 88 per cent. glycerin. After a period of forty-eight days the animal in question had a fit, became lethargic, and died eight days later, *i e*, fifty-six days after the inoculation. In a later paper McIntosh (1920) stated that he had transmitted the disease from this first monkey, in series, through two further generations in monkeys, and from an animal of the second generation of the series he had succeeded in infecting rabbits. It may be noted that in all the monkey experiments the incubation period of the disease was a very long one. In one case no symptoms had developed seventy-seven days after inoculation, which was then repeated, the animal becoming ill eleven days later and being killed sixty-eight days after the second inoculation. In the second passage experiment two monkeys were inoculated with the cord and basal ganglia of the last-mentioned animal, one of these (a baboon) became ill about four months later, and died 137 days after the date of inoculation. The result in the other animal was negative.

Early in 1920 Levaditi and Harvier succeeded in infecting a rabbit with the brain emulsion from a fatal human case of encephalitis, and subsequently, in his extensive monograph on the subject, Levaditi described in all two successful attempts to transmit the disease to rabbits by the inoculation of human brain material. In only one of these instances does it appear that the virus was successfully kept active by serial transmission, and it may be noted that in this case the patient suffered from marked febrile herpes. The disease in the rabbits was one of short duration, producing death in five to six days. After four rabbit passages Levaditi and his co-workers succeeded in infecting a monkey. The animal developed symptoms ten days after an intracerebral inoculation, and died on the twelfth day. Its brain

showed "typical lesions," and was virulent for two rabbits, which died with similar lesions. It may be noted that in no case did Levaditi succeed in directly infecting the monkey from human sources, although the experiment was tried upon fourteen animals. In Switzerland Doerr and his colleagues reported a similar successful inoculation with cerebro-spinal fluid in 1921, and two further successes with brain material in 1922.

The criteria for the successful transmission of the infection has usually been held to be the development of the nervous symptoms of lethargy and sleepiness in the monkey, and of less definite symptoms in the rabbit. Fits and paralyses and evidence of meningeal irritation have been noted in both animals. After death lesions have been found in the brains of the animals akin to those seen in the human disease. These consist, in man, in a diffuse mononuclear leucocytic infiltration, some nerve-cell degeneration of an inconstant type with neuronophagy of a not very marked description, and extensive perivascular infiltration with lymphocytes. In some cases a good deal of cerebral oedema is present. The perivascular accumulations are the most striking finding, and are the ones usually relied upon to establish a histological diagnosis. The appearances figured by McIntosh and Levaditi closely simulate these.

Levaditi and his associates, working with a single strain of virus (Souche C) derived originally from the human brain, and maintained by passage in the rabbit, and also with other strains, pathogenic for the rabbit but isolated from the nasopharynx and saliva, which they considered to be identical with the cerebral strain, built up a large mass of conclusions as to the nature and features of the virus. They showed, *inter alia*, that it was pathogenic for rabbits not only by the intracerebral route, that of choice, but also by the intraocular, intraneural, intratesticular and peritoneal routes, as well as by scarification of the cornea or of the nasal mucous membrane. They also produced its specific pathogenic effects by the injection of a sufficient quantity into the muscles of the neck.

According to these workers, nervous symptoms develop in the rabbit only a few hours before death, which supervenes some six

Date	Animal	Case	Material used	Route of inoculation	Result
30 4 20	A	I	C S fluid	Intraocular	Negative *
"	B	"	ditto	Intrathecal and nasal mucosa	Negative
31 5 20	C	II	ditto	Intraocular	Negative
16 7 20	D	III	ditto	Under nasal mucosa and sprayed in nose	Negative
12 2 23	E	IV	Fresh brain emulsion	Intracerebral	D septic meningitis
"	F	"	ditto	Corneal scarification	Negative
19 2 23	G	"	Brain 7 days glyc	Intracerebral	Negative
"	H	"	ditto	Intratesticular	Negative
23 4 23	I	V	Fresh brain emulsion	Corneal scarification	Negative
"	J	"	ditto	Intratesticular	Negative
"	K	"	Filtered ditto	Intracerebral	Negative
1 5 23	L	"	Brain 9 days glyc	Intracerebral	Negative †
1 10 23	M	VI	Fresh brain emulsion	Corneal scarification	Negative
17 11 23	N	"	Brain 16 days glyc	Intracerebral	Negative
17 12 23	O	VII	Fresh brain emulsion	Neck muscles and intraperitoneal	Negative
"	P	"	ditto	ditto	Negative
21 12 23	Q	"	Brain 4 days glyc	Intracerebral	Negative
31 1 24	R	VIII	Fresh filtered brain	Intracerebral	Negative
"	S	"	Ditto unfiltered	Neck muscles and intraperitoneal	Negative
10 3 24	T	IX	Fresh brain emulsion	ditto	D septic peritonitis
14 3 24	U	"	Brain 4 days glyc	Intracerebral	D 6 days ‡
"	V	"	ditto	Neck muscles and intraperitoneal	D septic peritonitis
17 3 24	W	X	Fresh brain emulsion	ditto	Negative
"	X	"	ditto	Intraocular	Negative
"	Y	"	Brain 18 hours glyc	Intracerebral	Negative

\* This animal was found to be paraplegic on the day following the injection. It was killed on the next day and an emulsion of its brain and cord injected subdurally into another animal, which remained well until killed eight months later.

† This animal developed pareses and inco-ordination five months later. It was killed when *in extremis*. The brain showed no lesions, and failed to infect four other rabbits by intraocular, intracerebral or intramuscular injection, either when fresh or after glycerination.

‡ No histological changes were found in excess of those seen in controls. A few focal hæmorrhages. Two other animals inoculated with the same material died of a septic infection.

to eight days after inoculation. The histological examination of the brain shows a diffuse mononuclear infiltration, some nerve-cell degeneration with neuronophagy, and extensive perivascular infiltration with lymphocytes. Polymorphonuclear leucocytes

play some part in the reaction in cases which evolve with unusual rapidity. McIntosh (1923) confirmed Levaditi's findings, with only a few differences in matters of detail, but unlike the latter, who held that the rabbit was with difficulty infected direct from the human subject although with readiness from another animal, he found such direct infection to be a matter of ease, and went so far as to advocate it as a diagnostic measure. The same suggestion was put forward by Thalhimer.

The writer of this book, on reading the account of Levaditi's successful transmission to rabbits in 1920, commenced experiments upon the same lines, and carried them on until 1924, when the source of material was cut off. Rabbits were used exclusively, and material from ten cases of the acute disease was investigated, all the advocated routes of infection being tried. The results are shown in the table on p. 142.

These experiments, which are a chronicle of entire failure, were very distressing to the author at the time, in view of the ease with which positive results seemed to be obtained by others. Douglas, in an outbreak in Sheffield in 1924, had a similar experience. The possible significance of these results was shortly to be reconsidered.

It had been observed by Levaditi and his associates that their passage strain of virus, inoculated by scarification of the cornea in rabbits, engendered an intense keratitis which was followed by the propagation of the virus to the brain and death of the animals with the lesions of encephalitis. This recalled a fact observed in 1910-11, by Gruter, that the fluid from febrile herpetic vesicles, inoculated upon the cornea of rabbits, produced a violent keratitis. It was found by Doerr and Vochting (1920) and abundantly confirmed, that the disease did not always remain a local one in the cornea, but was often succeeded by nervous symptoms, spasms, paralysis, torticollis, and the like. Death followed upon these manifestations, and it could be shown that the virus was then present in the brain, in which the histological changes were very similar to those described in the encephalitis experiments.

The problem which emerged from these varied findings shaped itself into the question of what was the relationship of the herpetic virus to that of encephalitis? Were the two distinguishable

viruses capable of producing similar results? Were the two viruses really one? or were the results obtained with encephalitic material false and due to a chance contamination with the herpetic virus?

Levaditi boldly stakes upon the second of these three possibilities, but claims that a slight modification of tissue affinity exists in the two cases, a strain of virus which causes encephalitis being more "neurotrope" than one which causes primarily herpes or keratitis, which is "dermotrope."

Reviewing the whole matter in the light of what has already been said, the dispute really narrows itself down to the infections which have been investigated in the rabbit. The monkey work is trivial, in so far as positive results go, and even then hardly comparable. McIntosh and Turnbull, and Loewe, Strauss and Hirschfeld each claim one success in the direct transmission of the malady from the human brain to the monkey, and Levaditi claims to have done this with a passage rabbit strain of the virus. The incubation period in the case of the first-mentioned workers was forty-eight days, and in their serial transmissions 68-116 days; in the case of the second group, one day and six days respectively, and in Levaditi's experiment ten days. It is hard to conceive that a single infection could be capable of such extreme vagaries, and a report by McIntosh of the spontaneous occurrence of encephalitis in one of his control monkeys may have quite a different significance to that attributed to it.

Incidentally these results in animals would effectually dispose of the suggestion that there was any identity between lethargic encephalitis and poliomyelitis, a fact also shown by the experiments of Amos (1921), who found no evidence of cross immunity between the two conditions.

Turning to the real bone of contention—the rabbit results—it may be noted that no differences, either in manifestations or in histological appearances, are to be found between the results of inoculation with the "encephalitis" virus and with that of febrile herpes. The work of Levaditi, and of Doerr and Schnabel, has further shown that there exists a crossed immunity between the two infections, so that they are to be judged as specifically the

same by the strictest of immunological criteria. Is then the same virus responsible for the two conditions, or have the positive inoculation results in encephalitis been due to the chance presence of the herpes virus?

The writer's own results lead him to the latter conclusion, since by no manœuvre whatever, including corneal scarification, has it been possible to produce any infection with human brain material. In many of the published experiments, upon which the view of an independent encephalitis virus is based, the strains in question have been obtained from the nasopharynx or from saliva, and deductions have been built up therefrom as to the route of infection and the carrier state. It is to be remembered that the results of Lowenstein and Levaditi show that the herpetic virus is an extremely common thing on the surface of the body, and it is also agreed that it is frequently present in normal saliva. The organism must therefore certainly be about the body in many cases of encephalitis lethargica, though it is not, as a rule, visibly active, for in ninety-two cases of the disease reported by the Ministry of Health (1922), in which some form of skin eruption was present, herpes only accounted for twelve of these. The total incidence of herpes in 1,278 observed cases was only a little under 1 per cent. On the other hand, in lobar pneumonia, in which febrile herpes is a classical sign, the occurrence of a true encephalitis would seem to be unknown.

The evidence of clinical observation, then, is that the herpetic virus does not tend to cause encephalitis in a disease in which it is almost constantly present and active and, on the other hand, that in encephalitis herpetic skin manifestations are likewise rare. Furthermore, it has been found that the encephalitic condition produces no immunity against herpes of the skin, even in convalescence. Nevertheless if, in view of the experimental results, we are to go so far as to suggest that in certain cases—rare it is true—the herpetic virus is present in the brain, not as an ætiological agent, but as a contamination, we are making what might be regarded as a very questionable assumption. The possibility of this has been made less remote by the very important observations of Flexner. With the incomparable facilities of the Rockefeller

Institute, and after failure in 1919 to induce the disease in monkeys at a time when successes were being claimed by Loewe and his associates, he had similar negative results throughout 1920, 1921 and 1922, when the rabbit was used as the experimental animal and material from some forty cases of encephalitis was examined. Finally in 1922-23, in conjunction with Amos, Flexner inoculated large numbers of rabbits intracerebrally with cerebro-spinal fluid, not only from cases of encephalitis, but from other conditions as well, making in all more than 100 injections. In only a single instance was a positive result achieved—and the fluid in this case did *not* come from a case of encephalitis<sup>1</sup>. In the one instance in which a transmissible virus developed in the rabbit, giving all the features claimed for the virus of encephalitis, the animal was inoculated with the cerebro-spinal fluid from a man with chronic neurosyphilis, who had never had encephalitis. The same fluid, examined at a later date, no longer showed the presence of the virus. This virus was capable of exciting keratitis by corneal inoculation as well as encephalitis by cerebral inoculation.

The obvious conclusion would appear to be that all the positive results which we have recorded have been due to the herpetic virus. There still remains, however, Levaditi's contention that the causal agent in encephalitis is merely a strain of herpetic virus which is endowed with a special neurotropic affinity. In examining twenty-six examples of saliva, the French savant found a virus in twenty-one of these which produced a pustular keratitis, akin to that produced by his encephalitic strain, but which did not give rise to cerebral involvement by spread of infection from the cornea. Levaditi regards this salivary virus as a normal saprophytic inhabitant of the mouth which exists within, or in close conjunction with, the cells of the squamous epithelium, and he further considered it to be identical in nature with the encephalitogenic virus on the grounds of crossed immunity; the cornea, inoculated with the less potent virus, being after recovery immune against infection with the more active type, a fact which had also been observed by Doerr and Schnabel. It is highly significant, however, that in two cases Levaditi certainly did find the more active type of virus in the saliva, which was both keratogenic and encephalito-

genic. This, however, he interpreted as being the encephalitis virus, and the infected subjects as carriers! In view of his own demonstration of the marked variations in virulence which might be found in the virus from such a source, a hard and fast categorical distinction of this sort seems a little forced.

The thesis of an essentially neurotropic nature of the herpes-encephalitis virus, in the latter condition, has been very largely demolished by the work of Flexner and Amos (1925), who have shown that strains of the virus isolated from unequivocal cases of febrile herpes may be every bit as neurotropic as the accepted encephalitic strains. Such a virus, isolated by these investigators, reached the brain whether it was implanted upon skin or cornea, or injected into blood or testis, and was, in their hands, actually more strongly neurotropic than Levaditi's original cerebral strain (Souche C.)

The histories of these experimental infections originated at different times and in widely divergent spheres. Their lines of investigation have approximated and finally merged. No distinction can be drawn between the viruses which have occasionally been isolated by workers at encephalitis and those isolated with great frequency from herpetic vesicles, the saliva, and from time to time from other sources in human subjects not suffering from encephalitis. The conclusion is that the herpetic virus is a very widely disseminated infective agent in man, and it has been this which has contaminated the tissues used as an inoculum in the investigation of encephalitis.

It must be finally said that it is extremely doubtful if the condition of human epidemic encephalitis has ever been transmitted to the lower animals by the inoculation of brain material.

### THE ENCEPHALITIZOON CUNICULI

The already complicated question of the experimental investigation of encephalitis lethargica became still further involved as a result of the communications emanating from Kling, Davide and Liljenquest, in Sweden, in 1921. They reported the successful transmission of the disease to rabbits, not only with brain substance

but with filtered nasopharyngeal and faecal materials. Their results also differed markedly from those of others in that the experimental disease evolved only months after the inoculation, was chronic in nature, and did not always produce death of the rabbit, the essential lesions being discovered only when the animal was killed. The lesions themselves also differed in character from those noted by previous writers, in being nodular and necrotic in type, as well as perivascular. These histological differences were very definite. Furthermore their virus, although transmissible in series from rabbit to rabbit, did not cause keratitis when inoculated upon the cornea.

Here, then, was an entirely different type of disease coming from Sweden. After a short and highly polemical discussion, and the interchange of material, Levaditi (1928) announced that this condition was not the result of any encephalitic virus of human origin at all, but the consequence of invasion of the rabbits by a protozoon parasite which, together with the effects resulting from its presence, was at the time in process of being described by workers in different countries.

This parasite, which had actually been seen by Doerr and Zdansky in material which had been supplied to them by Kling, and upon whose nature they had speculated, was first described by Wright and Craighead (1922) in America. They found it to be the causal agent in a type of infectious paralysis in young rabbits and observed its presence in tissues other than the central nervous system. It was also independently discovered by C. C. Twort, who noted the existence of lesions in the kidney as well as in the brain. The condition was described by Twort as one of spontaneously occurring encephalo-myelitis and nephritis, due to a filter-passing virus. It does not appear, however, that the direct filtrability of the infecting agent was demonstrated. At about the same time McCartney cut sections of the brains of some 800 rabbits, which had been sacrificed at the Rockefeller Institute for various purposes, and demonstrated the presence of chronic inflammatory lesions in about a half of them. These were similar to those described previously by Bull (1917) and Oliver (1922), who had encountered them in the course of other investigations. The

lesions in all cases were mononuclear, nodular, necrotic and perivascular, and appeared to be identical with those observed by the Swedish workers.

The life-history of the organism which Levaditi has termed the *Encephalitozoon cuniculi*, which appears responsible for many of these deceptive appearances and which certainly constituted the source of error in the Swedish work, has been enquired into by Levaditi, Nicolau and Schoen (1924), who were able to demonstrate the presence of microsporidial cysts in the brains of the infected animals. These were often far removed from the inflammatory foci and were not themselves always associated with reaction. The rupture of the cysts, and the freeing of the protozoan spores, resulted in the formation of granulomatous masses and the phagocytosis of the spores by macrophages. They were further able to identify the various inflammatory lesions described by Bull, Oliver, Twort and the Swedish workers as being all associated with this parasite, and to show that the "encephalitis" virus of Thalhimer, whose work was closely associated with that of Loewe and Strauss in New York, was also contaminated with this protozoon. McCartney was also able to find the parasite in his Rockefeller material.

The parasite is pathogenic not only for rabbits, but also for rats and mice, will infect these animals when inoculated by any route and produces lesions in the liver, spleen, kidneys and brain, its maximum effects, however, fall upon the last two organs. It is transferable from animal to animal by serial inoculation and, being excreted in the urine, is apt to become endemic in animal houses and breeding establishments. It is by no means so universal a contaminant as might be imagined from the literature. The French workers do not appear to have been troubled by its presence, and in the laboratory in which the writer worked upon encephalitis (Manchester), both the parasite and the lesions which it occasions were conspicuous by their absence.

The spores of the organisms can be demonstrated in smear preparation, either in the fresh state or by staining after fixation in the liquid of Bouin-Brazil. The spore envelope appears somewhat impermeable to ordinary stains, but the effect of such

fixatives is to modify this and permit the spores to be stained by Unna's polychrome or by Mann's stains. A similar result can also be obtained by treatment with 5 per cent hydrochloric or sulphuric acid which enables the spores to be coloured with iron hæmatoxylin.

The free spores are small oval or semilunar bodies, measuring

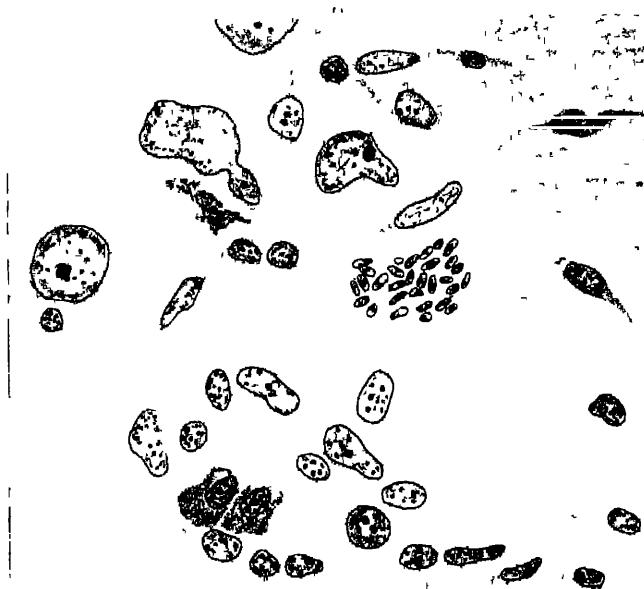


FIG 11 —Section of rabbit's brain, showing a cyst containing spores of *E. cuniculi*, and some inflammatory cells in the vicinity — (After Levaditi, Nicolau, and Schoen)

$2.5 \times 0.5-1 \mu$ . The cysts, which are met with both in brain and kidney, are much larger, round or oval bodies, measuring  $20-80 \mu$  in diameter and packed with parasites (Fig 11). The lesions in the kidney are most marked in the medulla, where small granulation nodules are formed which are exactly similar to those found in the brain. The parasites are to be seen in large numbers within the epithelial cells. By the bursting of the cysts

the spores are set free, reach the lumen of the tubule and pass to the exterior.

Before leaving this subject it may be well to say a word of warning about the interpretation of certain cerebral lesions, both in man and in inoculated animals. The most clamant changes in human encephalitis, and in all these animal experiments—the changes upon which most attention is inevitably focussed—are the perivascular, mononuclear-cell, accumulations. These are in no way specific for any known type of disease. They merely represent a common picture, in any chronic inflammatory or irritative cerebral condition in which the cellular response is a mononuclear one and in which, on account of the peculiar anatomical arrangements of the brain, the cells accumulate in this special position. The picture is seen in encephalitis, in which the lesions are usually most pronounced in the brain stem and basal ganglia, although this distinction is not invariable, and I have seen them very much in evidence in the cervical cord in a case of Professor Ryrie's. They are also seen in paralytic dementia; in the vicinity of gummata; in sleeping sickness, and in the trypanosomiasis of animals. to mention only a few of the possible conditions. The same type of lesion is, of course, present in the cord in poliomyelitis, although the collections here are smaller, in conformity with the lesser size of the vessels, and in the acuter cases the polymorphonuclear cells participate. It should therefore be remembered that in any inflammatory condition in the central nervous system cellular accumulations may occur in the perivascular spaces and that these are without specific significance.

The finding of isolated foci of mononuclear cells is also to be regarded as non-specific and to be treated with caution. Quite apart from the very gross lesions described by McCartney and others, and associated with the microsporidial parasite of rabbits, the brains of these animals sometimes show isolated mononuclear foci when apparently normal in every other respect. These lie close to the ependyma of the anterior horns of the lateral ventricles and in the almost contiguous grey matter of the caudate nucleus; they are much less frequent in other positions. The writer has found these collections in seventeen out of twenty rabbits' brains,

and occasionally they have been associated with slight perivascular accumulations in the immediate vicinity. The appearance of foci of mononuclear cells in this situation is so constant as to be looked upon as normal.

Another point, which may be considered, is the significance and interpretation of nervous symptoms in rabbits. These animals develop paraplegia with great readiness when subjected to injections and infections of all kinds which, as far as is known, is a non-specific manifestation. Other less clamant nervous symptoms may therefore probably be due to similar causes. The spontaneous development of extensive palsies in rabbits is familiar to most persons who have much to do with them. The writer examined a number of animals suffering from this condition in a search for the *Encephalitozoon cuniculi*, which he failed to find. The brains of such animals did not show any inflammatory lesions, and transmission experiments failed, except in one case in which paralysis supervened after an incubation period of seven days, here, however, further propagation of the malady by intracerebral, intramuscular or intra-ocular inoculation of brain emulsion failed.

It cannot be over-emphasised that the investigation of nervous diseases by rabbit inoculation should be prosecuted with great caution as regards the interpretation of any results achieved.

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## CHAPTER VIII

### ULTRAMICROSCOPIC AND FILTER-PASSING VIRUSES (*contd* )

#### RABIES

THIS disease has certain definite analogies with the other virus diseases of the central nervous system, which are discussed in detail in Levaditi's imaginative but suggestive monograph "Ectodermoses Neurotropes," to which the interested reader is referred. The malady is the earliest of the diseases, believed to be due to a filtrable virus, to have yielded any substantial part of their secrets to the experimental investigator; for, as is well known, it was Pasteur himself who, having demonstrated the presence of a virus in the central nervous system, and failing in all attempts to cultivate it outside the body, resorted successfully to the method of carrying it on by propagation in the brain of the living animal. Except in matters of detail, little has been added to our knowledge of the disease as Pasteur and his school left it

The filtrability of the virus has been a matter of some dispute, but is now generally accepted. The most definite experiments upon this point are those of Poor and Steinhardt (1913), who obtained diffusion into glycerin of the virus from the salivary glands of a rabid dog, and found it to pass Chamberland F filters readily. Others who have obtained very irregular results have worked chiefly with brain substance. It would seem that if any doubts upon this score remain they should be resolved by experiments upon saliva, and not upon brain extracts, whose clogging effect upon filters is very marked. The infectivity of the saliva is very much greater than that of the brain, and it is now realised that this is not limited to the period in which the animal displays symptoms. Rabineaux and Guinard, by means of a salivary fistula, were able to show that the virus was present in the secretion four days before the symptoms declared themselves, and clinical observations have shown that in certain instances this has been

the case as long as fourteen days before the disease became apparent. On the other hand, it has also been shown that in the very rare instances in which an animal recovers from the disease the infectivity of the saliva persists for a variable period, so that an actual carrier state is possible.

Perhaps the most important contribution to our knowledge of rabies, in the post-Pasteurian period, has been the important discovery by Negri, in 1903, of the intracellular formations which bear his name, and which he believed to be a protozoon parasite in a certain stage of its evolution. The findings of Negri have been abundantly confirmed and a quick and reliable method of diagnosis put into our hands. Upon his interpretation of them opinion has been widely divided

Two views have been put forward. The one, that of Negri, regards these bodies as specific living parasites, the other looks upon them as degeneration products, the result of the disease or, at the most, comparable to other cell inclusions whose nature is uncertain, but which, as a class, are not usually regarded as parasites. The arguments in favour of the latter conception are, briefly; (1) The absence of these bodies from the saliva of the rabid animal, which is highly infectious, and to a large extent also from the medulla oblongata, in which as is well known the virus is particularly active, whilst they are present and best developed in parts of the brain which are not so highly infective. (2) Their absence from the brain in stages of the disease in which the virus is already present. (3) The absence of the Negri bodies in *virus fixæ* (4) The filtrability of the rabies virus, and the fact that it will diffuse from the brain into fluid media and glycerin (Remlinger, 1919). The upholders of the parasitic theory have maintained it chiefly upon the grounds that the Negri bodies are found solely in rabies, and never in other diseases of the nervous system, that they are present in cells which in other respects appear healthy and are therefore not likely to show degeneration products; that their structure is constant, characteristic, has tinctorial affinities with the protozoa, and undergoes certain changes in form suggestive of developmental phases.

Life cycles of varying degrees of completeness have been

described by a number of workers from Negri onwards. He himself looked upon the parasite as a protozoan and accepted for it the name *Neurocytes hydrophobæ*. Volpino (1904) suggested that at one phase in its life-history the parasite must be ultramicroscopic, and Anna Williams and Lowden (1906) classified it as a microsporidian and suggested analogies with *Nosema lophii*. They considered that they could detect forms so small as to be probably filtrable and concluded that the evidence in favour of the Negri bodies being protozoa was overwhelming. The existence of minute types of Negri bodies has been commented on by a number of other workers who have noted their presence in *virus fixæ*. Levaditi asserts that these are non-specific granulations and have no relationship to the Negri bodies.

In 1913 Noguchi, following the technique used for the cultivation of the globoid bodies of poliomyelitis, announced that he had cultivated the virus of rabies. This work has not been confirmed.

The problem of the nature of the Negri bodies received a fresh stimulus in 1924 at the hands of Manouélian and Viala, of the Pasteur Institute. They observed in them certain changes, which they interpreted as representing their degeneration and disappearance. To follow as far as possible the development of these bodies they applied themselves to the study of the earliest phases. By using a rather complicated technique for fixation and embedding, and overstaining with Mann's fluid followed by differentiation, they discovered appearances in their sections of brain material which they interpreted as being due to a protozoal parasite. These consist in the presence of small oval bodies, whose appearance in the published plates is indistinguishable from that of the spores of Levaditi's *Encephalitozoon cuniculi*, and which varied in size from 1-2  $\mu$  down to the limit of visibility. These bodies were newly discovered formations, not visible by the more ordinary methods of staining, and distinct from the Negri bodies, although showing relationship to them through transition forms. They concluded that the Negri bodies were composed of agglomerated masses of these small parasites, at first free in the cell but later becoming fused and undergoing degeneration.

They also claimed to have demonstrated their parasite in the

secreting cells of the salivary glands, in which the Negri bodies are not found, and in the glandular ducts. They believed that the parasite was of a type similar to the *Encephalitozoon cuniculi* and designated it *Encephalitozoon rabiei*.

The latest contribution to the subject comes from Levaditi, Nicolau and Mlle. Schoen (1926) who, impressed by the similarities between the Negri bodies and their *Encephalitozoon cuniculi*, took up the study of the former in great detail. They conclude that the disease is due to a microsporidial parasite, invading the brain and excreted by the salivary glands. They lay some stress upon the fact that in other species comparable microsporidial parasites are known, and instance the *Nosema bombicis*, which is causal of the disease pébrine in silkworms, and the case of *Lophius piscatorius*, in which the parasite, *Glugea* (*Nosema*) *lophii*, forms sporocysts within the cells of the nervous system. It is, however, so far a cry from these cold-blooded lowly forms of life to the mammalia that the analogy can hardly be considered a striking one. For Levaditi and his colleagues the Negri bodies are microsporidial cysts (pansporoblasts), and represent only an isolated stage in the life cycle of a protozoon which, in other phases, is so small as to be filtrable, it is in this phase that it is eliminated by the salivary glands. The parasite is referred to as *Glugea lyssæ*.

Levaditi resolves the difficulties which we have recounted in the acceptance of a protozoon parasite as follows. The organism invades the tissues in the minute phase, in which it is found in the saliva, and is propagated along the peripheral nerves to the central nervous system. In the latter the tendency is for a further intracellular development. This only takes place in regions and under circumstances where a sufficiently long sojourn within the cell is possible. In the medulla and pons the resistance of the nerve cells is stated to be slight, the cells degenerate and undergo neuronophagy and the developmental cycle of the parasite is never completed. On the other hand, in the cells of the hippocampus major, the cortex cerebri, and in the Purkinje cells of the cerebellum, resistance is more marked and the cells remain intact, allowing the parasite to develop to the pansporoblast stage. This suggestion is compatible with the well-known fact that the Negri

bodies develop in cells whose appearance, except for some fragmentation of their Nissl's substance, is otherwise healthy. Another difficulty, the practical absence of Negri bodies from *virus fixe*, is explained as being due to the greater virulence of the infection in this case, which brings about the decease of the animal before sufficient time has elapsed for the development of pansporoblasts.

By fixation of the materials in Bouin-Brazil, and staining by a slow Giemsa method, the earliest stages of the intracellular phase appear to be the development, within the nerve cells, of small rounded bodies with a central chromidial mass which have a hyaline capsule. The thickening of the capsule as development proceeds renders the contents stainable with difficulty by basic colours and causes the resulting Negri bodies to stain in the familiar, more or less hyaline, fashion.

Levaditi has not succeeded in demonstrating anything approaching a convincing life cycle, and although of necessity well aware of the findings of Manouélian and Viala, he does not discuss these. Since, however, he states that his *glugea* parasite cannot be considered in any way identical with the *Encephalitozoon cuniculi*, and definitely decides against classifying it with the microsporidia, it is clearly evident that he is not convinced of the correctness of the results of his colleagues. Furthermore, the appearances figured in his papers in no way resemble those shown by Manouélian and Viala.

The question of the nature of these supposed parasites was one submitted to the International Conference on Rabies, convened by the League of Nations, at the Pasteur Institute in 1927, where the matter was fully discussed. The commission did not, however, feel itself in a position to make any definite pronouncement upon the subject.

Some further interesting properties of the rabies virus, which have a considerable practical bearing, have been brought into notice by recent work. It has been long suspected that the *virus fixe*, although of exalted pathogenicity for rabbits, had suffered considerable alterations in other directions and has become comparatively avirulent for man. This belief has led to many

modifications of the original Pasteurian method in the direction of eliminating from the treatment the use of the more attenuated cords, the earlier use of the more active cords, especially in cases of severe bites and those about the face, and the use throughout of fully virulent cords, variously modified. Some experiments of Levaditi have shown that even in the case of the rabbit the *virus fixe* is relatively non-pathogenic, when the inoculation is made subcutaneously. Out of ten rabbits inoculated in this way with *virus fixe* one only contracted the disease, whilst out of seventeen inoculated in like fashion with street virus, twelve became rabid. Marie showed, and Levaditi confirmed the fact, that fresh *virus fixe*, inoculated subcutaneously into monkeys, the animal most nearly related to man, did not cause rabies, although fully virulent by subdural inoculation; and Nitzsch on one occasion inoculated himself subcutaneously with the same material without suffering ill effects. Remlinger also, owing to a laboratory mistake, inoculated a human subject with two centimetres of the cervical portion of a cord only forty-four hours old, without mishap. He repeated the experiment with two rabbits, and failed to cause any infection in these when the virus was introduced subcutaneously.

It would therefore appear indubitable that the *virus fixe* of Pasteur has suffered considerable modification of its properties, in such a way as largely to have lost its ability to reach the nervous system from the periphery, although it follows, from the results of intracerebral inoculation, that it is fully capable of producing its specific damage when directly introduced therein.

The innocuousness which it displays in practice is consequently due not only to its attenuation by drying, dilution, etc., but also very largely to this inability to invade the central nervous system when injected subcutaneously.

Levaditi regards the *virus fixe* as representing an irreversible mutation in the infective agent, a mutation which betrays itself most characteristically in the experimental animal by the loss of ability to form pansporoblasts (Negri bodies). His experiments show that various strains of street virus vary much in the readiness with which they undergo the change to *virus fixe*. Some forms

reach an incubation period of seven to eight days, and show no Negri bodies, as soon as ever they are introduced into the rabbit's brain. Others, as in the classical strain of Pasteur, show a progressive shortening of the incubation period to the fixed minimum and only lose their ability to form Negri bodies after a number of passages; whilst yet other strains show no tendency to assume the characters of fixed virus. Their incubation period varies in uncontrollable fashion, from experiment to experiment, and Negri bodies continue to be formed although passage is persisted in for a long time

The considerable differences in the experimental behaviour, not only of different strains of street virus but also of *virus fixe*, was one of the problems discussed by the recent International Conference upon Rabies. The matter is one of more than academic interest, since it has been felt that certain failures of the Pasteurian inoculation may be due to immunologically differing strains of virus. A. C. Marie discussed these remarkable differences in properties, mainly concerning virulence, which have come to light amongst different strains of street virus. He especially instanced the Koritschoner strain, which was obtained from the brain of a man dying twenty-four hours after the inoculations which had been given for protection against the bite of a rabid animal. At post-mortem the brain lesions resembled rather those of meningo-encephalitis than rabies, whilst the symptoms developing in animals which were inoculated with this brain were of the same type, death occurring in three or four days. However, since there were definite crossed immunity effects between anti-rabies serum and the Koritschoner virus, as well as between an antiserum prepared from this virus and a classical strain of *virus fixe*, its identity with rabies would seem probable. Marie also instances, as another example of such variations, that Bouffard, in Senegal, has recorded that canine rabies exists there in a form in which it is not transmissible to man, although fully virulent for laboratory animals. Marie is of the opinion that such differences between strains of street virus are reflected in the different strains of *virus fixe* developed from them, which his report discusses. These differences, which bear no relationship to the number of passages

which the virus has suffered, are well illustrated by the effects of the subcutaneous inoculation of the virus into rodents. Whereas the Paris strain produces a mortality of 20–30 per cent. in these animals, and the Roman and Turin strains 60 per cent., the strains in use at Palermo and in the Russian institutes of Cracow, Kieff and Odessa, produce rabies in 100 per cent. of cases ; one in particular, that of Sassari, being still active in this respect even in dilutions of 1 in 30,000. Such variations are hard to explain without the conception of different biological strains, and raise at once the question of the desirability of using a polyvalent vaccine instead of one representing a single strain of virus. This, however, seems to be the only evidence tending in this direction, and all these viruses, whatever their source and activities, are neutralisable by a single anti-rabies serum. The matter is recognised to be one for further research.

The question of paralytic accidents in the course of anti-rabies treatment is one which has excited great interest, it being held by some that these are rabid phenomena and by others that they are non-specific. Remlinger has investigated over 400 such cases and finds that they are of three main types . paralysis of the Landry form, which has a mortality of 30 per cent. ; dorso-lumbar myelitis, with a mortality of 5 per cent. , and facial neuritis, which is non-fatal. An examination of the relative frequency of these complications in the different methods of immunisation shows that in the treatment of 41,081 persons at the Institut Pasteur of Paris, only eight cases of such paralyses have occurred, all of which recovered. The Pasteurian method and its modification by Calmette, in which the non-virulent cords are excluded and the dried virulent cords preserved in glycerin, in which they only slowly deteriorate, are fairly free from such sequelæ, as is also the Hogenes method. The heating method of Puscariu, on the contrary, seems rather prone to such accompaniments. It is highly desirable that in cases in which such paralyses terminate fatally a thorough search for the rabies virus should be made in the brain of the subject in order to throw as much light as possible upon the nature of the condition, since although it is generally regarded as avirulent for man by subcutaneous inoculation, the living *virus fixæ* does

not seem wholly innocuous or its use devoid of risk. One of the questions considered by the International Conference on Rabies was the efficaciousness of the killed virus, which is free from the risks of the living, infinitesimal though these may be. The general conclusion was that the immunity provided by the living *virus fixe* was superior to that produced by phenolised and otherwise killed virus, and that the figures of the institutes using the former were in general slightly the better of the two. Nevertheless the fact remains that a certain risk, however slight, is attached to the use of a living virus, and the Conference recommended to its conveners the desirability of arranging for a large scale trial of the two methods under comparable conditions.

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## VACCINIA—VARIOLA

The increased attention lately given to diseases of the filtrable virus series has caused a revival of interest in this infection.

The maladies of the pox group include vaccinia, variola and its closely related phase of paravariola or alastrim, sheep pox, horse pox, goat pox, swine pox and, probably, the pustular stomatitis of horses, which in this country and in France is commonly considered to be the same infection as pox. A contrary opinion is held in Germany. Of these maladies only two, human pox and sheep pox, are dangerous generalised diseases, the others all being local eruptive processes. Sheep pox and goat pox are independent maladies, having no epidemiological or immunological relationship with the other diseases of this type ; but horse pox, cow pox and human variola have long been recognised as forming a group, largely interchangeable and capable of increased adaptation to one or other of the species.

**Nature of the Virus.**—Most of the work has been carried out with vaccine-virus, either in the form of calf lymph or of lymph obtained from rabbits. The filtrability of the infective agent is disputed, and though a number of reports of the virus passing Berkefeld filters have been forthcoming, the general opinion in this country seems to be against any ready acceptance of its filtrability. Gordon (1925) found that Berkefeld filtrates of lymph were entirely devoid of activity towards animals, and had at the same time lost all power of reacting with specific antisera. It may be that here, as is also possibly the case with the herpetic virus, the active agent is in combination with cellular material, and on this account unable to pass the filter. This is suggested by certain results which go to show that its filtrability is enhanced by the autolysis or digestion of the lymph. Levaditi (*loc. cit.*, 1928) claims that the virus readily passes collodion sacs which to a large extent keep back complement, hæmolysin,

and diphtheria toxin. If this should prove to be the case it becomes certain that its failure to pass the coarser bacterial filters is due to combination with or adsorption upon relatively large particles such as abound in vaccine lymph.

**Properties of the Virus.**—The investigation of vaccinia is rendered easy by the fact that the rabbit is a susceptible animal. It is in this respect that the most striking difference between vaccinia and variola emerges, since the latter is non-pathogenic for rabbits. The reaction in the rabbit has been widely studied, and the possibility of quantitatively estimating the virulence of a given preparation of virus by this means has been firmly established. Immunity to vaccinia in this animal begins to become generalised on or about the fourth day after inoculation, and becomes universally established by the seventh day. Its duration has been variously estimated by different observers, and must depend a good deal upon the circumstances of the tests, but it may be said in general to persist for from five to seven months.

The vaccinia virus by its development in the body occasions antibody formation. Three types of antibodies have been studied : neutralising, precipitating and complement-binding. The neutralising antibody confers upon the serum of the vaccinated animal the power of neutralising the vaccinia virus *in vitro*, and a like property is present in the serum of convalescents from small-pox. This serological property is a relatively crude index of immunity, since it fades and can no longer be demonstrated long before the accompanying immunity is lost. Re-vaccination results in the reappearance of this substance in the blood.

The presence of specific precipitating substances in the blood of vaccinated subjects, and of persons suffering from variola, has been remarked upon by a number of workers. Torikata (1917) found that a boiled and filtered extract of vaccine lymph had antigenic properties, and on injection into an animal resulted in the production of immunity and a specific precipitating serum. These observations were extended by Tomarkin and Suarez (1917), who found that extracts of lymph heated to 100° C for fifteen minutes gave better reactions *in vitro* than did unheated extracts. The reaction was obtained both in the case of animals inoculated with

calf lymph and with vaccinated human subjects. Gordon, in his recent investigations of the viruses of vaccinia and variola, has made extensive use of a reaction of this nature, between immune serum and a suspension of calf or rabbit lymph freed from gross particles by light centrifugalisation. The reaction consists in a well-marked agglomeration of the particles of the suspension, which he designates agglutination. He found this test of considerable use in determining the presence of the virus in infective materials.

Gordon further found that the serum obtained by injecting rabbits with vaccinia virus agglutinated not only suspensions of vaccine lymph, but also those prepared from confluent small-pox scabs, as well as alastrim scabs from five different outbreaks; no such effect was produced with scabs from chicken-pox, Henoch's purpura, or with pus.

Complement-fixing antibodies have been the subject of even more numerous observations, since the finding by Jobling, in 1906, of complement-binding antibodies in the serum of vaccinated calves. Later Sugai described the same antibodies in the blood of small-pox patients, and was able to obtain fixation both with antigens of calf lymph and of variolus pustular material. Kolmer investigated the subject extensively in 1916, using serum from small-pox cases as well as from vaccinated persons and animals, and antigens of vaccinia virus and extracted small-pox scabs. He found that a majority of cases of clinical small-pox gave a positive reaction, and that this was obtained with both antigens. The sera of rabbits inoculated with cow-pox virus also yielded positive complement-fixation results with both cow-pox and small-pox antigens from the seventh to eight day after vaccination. Gordon also has used the complement-fixation test and has obtained satisfactory fixation between anti-vaccinia serum and antigens of calf lymph, alastrim scabs, and confluent small-pox scabs. Controls with other materials, including varicella and brain material from a case of encephalitis lethargica, were negative.

The presence of such well-defined antibodies in the blood brings into prominence the possibilities of serum therapy and the use of the serological tests in diagnosis. In their present form their

utility for the latter purpose is not likely to be great, since the tests are usually negative in early and mild cases in which help in diagnosis is especially needed. With regard to treatment, all energies in the past have rightly been concentrated upon the preventive side of variola, but, with the present breakdown of the enforcement of this, specific therapy may become increasingly important. Teissier and Marie have employed serum from convalescents in the treatment of cases of small-pox and claim favourable results, and many observers have shown the possibility of producing passive immunity against vaccinia by the use of immune serum.

**The Relationship of Variola and Alastrim to Vaccinia.**—As Gordon points out in his recent review, the existence of small-pox of a mild type is no recent development, as is believed in some quarters, but was perfectly described in epidemic form in Gloucestershire by Jenner, whose description would stand well for what at the present time is often, and rather unfortunately, called alastrim. Paravariola would be a better term if a specific one be needed. It was doubtless, too, the recognition of such mild forms which originally led to the practice of inoculation.

The matter has recently been reviewed by Ledingham (1925), who points out that most of the strains of calf lymph now commercially manufactured were undoubtedly originally derived from human variola. As a rule the calf, like the rabbit, is insusceptible to direct inoculation with variola virus, but after passage of this through a monkey, and perseverance in the passage of material from the initial trivial lesions which are produced in the calf, adaptation eventually occurs and typical lymph pustules are produced. The most important direct observations upon the identity of the experimental results produced by materials from small-pox and alastrim are those of Blaxall, who obtained identical lesions in monkeys by the inoculation of alastrim virus and that of virulent small-pox. Attempts to directly infect the calf with both viruses failed, but in each case, starting with monkey material and effecting passages from calf to calf from the sites of apparently abortive inoculations, typical vesicular eruptions were eventually produced with both types of virus. Blaxall also carried out cross

immunity experiments with monkeys, which showed that material from English alastrim gave substantial protection against vaccinia. He further found that the variolised calves, mentioned above, were completely immune to vaccination. Ledingham concludes his immunological review with the statement that the mild form of small-pox at times manifest in this country "is merely a variant of variola whose toxic properties have become suppressed whilst its affinities for other animal species have not appreciably changed."

Gordon applied serological tests to material from Gloucester alastrim and virulent small-pox, and found that each gave specific reactions by complement fixation and his agglutination technique with anti-vaccinal serum. No difference could be detected by serological methods between alastrim and the confluent type of small-pox; and the antibodies produced by the injection of vaccinia virus appeared equally specific for this substance and virus-containing material from the other two infections.

**Attempts at cultivation of the vaccine virus.**—Noguchi showed that by means of intratesticular inoculation, and transference in this way from rabbit to rabbit, the vaccinia virus could be obtained free from extraneous organisms such as are commonly present in vaccine lymph. Attempts at the cultivation of the organism *in vitro* have not succeeded up to the present time. Endeavours have been made to produce growth of the virus in tissue cultures, and this seems to have been definitely accomplished by Parker and Nye (1925), who started with the bacteria-free virus obtained by intratesticular inoculations and, from the small tubercle-like foci there developing, carried on the virus in tissue cultures from normal testes for as many as nine generations. The results showed a definite increase in the amount of virus present, since on testing the cultural material on the cornea no result was obtained after a few days' incubation, but a positive reaction occurred after more prolonged cultivation. Carrell and Rivers (1927) found the cultivation of the virus in growing chicken-embryo pulp an easy matter. The tissues and extract containing the virus were left in contact, either at ice-chest temperature, or in the incubator, for a short period to allow of the virus becoming fixed to the tissues, which were then cultivated in the usual

fowl-plasma coagulum. At the end of eight days, or longer, the culture mass was withdrawn, disintegrated in a mortar, and found on testing to show an increase in vaccine content which was sometimes over a thousandfold. These authors considered the method one of possible commercial application. The chief difficulty in all work of this sort has been the avoidance of bacterial contamination, which causes liquefaction of the plasma and death of the cultures.

In connection with the same method it may be noted that M. Findlay (1928) has cultivated the virus of fowl-pox through four generations in tissue growths of chick embryo. He obtained the interesting results that the virus failed to survive in cultures of rat or mouse embryo, which would indicate a very sharp species specificity for this virus.

**The Vaccinia Virus and the Central Nervous System.**—Although attention has been very generally focussed upon the surface reactions to vaccinia virus, and epithelium would appear to be an especially favourable medium for the production of its characteristic effects, it has long been known that its lesions are not restricted to this tissue, and Ledingham believes, as a result of his researches, that the tissues primarily implicated are cells of the reticulo-endothelial system, which are involved in the production of a lesion which is essentially an acute infective granuloma; the involvement of the epithelial elements in this view is secondary, the virus having no special epidermal affinity. Ledingham looks upon the occurrence of skin-lesions in shaved areas, following upon the intravenous injection of vaccine in the rabbit (Calmette-Guérin phenomenon), as being due to local circulatory disturbances favouring the settlement of the virus in the dermal spaces. The anatomical arrangement of the dermal capillaries, described by Krogh, offers, in Ledingham's opinion, a favourable seat for the growth of any virus present in the blood or lymph streams, which is accentuated in locally irritated areas. With regard to other organs, it had been previously recognised that the testicle underwent inflammatory changes upon the introduction of vaccinia, but the credit for demonstrating the specificity of these must rest with Noguchi (1915). The adaptation of

the virus to the testicle is not immediate, but progressed gradually. After some time in the organ, a high concentration of virus is obtained, which reaches its maximum on the fourth or fifth day after inoculation.

A. Marie, in 1920, showed that vaccine lymph was virulent for the rabbit on intracerebral inoculation, causing a form of encephalitis fatal in from four to seven days. The whole of the central nervous system is invaded by the virus and becomes virulent for further animals, so that the condition can be propagated in series. According to Marie, with its habituation to the central nervous system, the virus loses its ability to affect the skin. Levaditi and Nicolau took up the subject with great thoroughness and found that the successful inoculation of the brain is not easy to accomplish, and can best be attained by the preliminary passage of the virus through the rabbit's testicle. Once established in the brain the virus, in their experience, showed an initial tendency to become ineffective, but after renewed testicular passage it recovered its virulence and ultimately could be propagated from animal to animal with the same ease as the rabies virus. The infected brain constitutes a type of vaccinia virus which has been called "neurovaccine," and has the great advantage, for experimental work, of being free from contaminating organisms. They found that it remained capable of setting up vaccinia when inoculated into the skin of susceptible animals, but appears somewhat less potent in this respect than the ordinary vaccine which has been cultivated continuously upon the skin (dermovaccine). This difference is especially seen in the fowl, where although no eruption of vaccine pustules is observed on inoculation with neurovaccine the animal becomes refractory to a subsequent inoculation with dermovaccine. In other essentials the characteristics of neurovaccine are similar to those of the ordinary vaccine lymph, and it has been used with success by Levaditi and his associates for the vaccination of the human subject.

As a corollary to his general investigation of the properties of the vaccine virus, and its sites of localisation in the body, Levaditi develops an elaborate argument for its affinity for tissues of ecto-

dermic origin, thus bringing vaccinia within a group of conditions, designated by him "Neurotropic Dermatoses," which includes rabies, poliomyelitis, lethargic encephalitis and heipes; all similar in some of their properties and all, according to this view, characterised by a special affinity for epidermis or for tissues derived from the ectoderm. This thesis is supported by some ingenious and interesting experiments, but on the whole, though serving to bring out undoubted similarities, it appears forced and rather inconclusive.

The possibility of experimentally implanting the vaccinia virus in the brain is of significance in connection with the occasional occurrence of post-vaccinal encephalitis. This was first noted by Turnbull, in 1912, in a boy of fifteen who developed cerebral symptoms after vaccination. Since then the condition has been recognised by a number of workers, and in this country Turnbull and McIntosh (1927) have examined and investigated eight cases at post-mortem. The account here given owes much to their work. The disease usually commences ten or twelve days after vaccination and is ushered in with headache and vomiting. Drowsiness, delirium, rigidity of limbs, pareses, paralyzes and coma may follow, and death occurs in a considerable proportion of cases after an illness lasting about 14-17 days. Recovery may take place even after paralyzes have declared themselves, and is usually complete. The gross post-mortem findings in the central nervous system are slight, and consist in leptomeningeal congestion and œdema, occasionally some softening of the brain and rare punctiform hæmorrhages. Histological examination shows perivascular infiltrations and large areas of perivascular softening and demyelination in the white matter, the latter being an especially characteristic feature of this condition. The changes are diffuse, but are most noticeable in the region of the pons and upper part of the medulla. The cord is also affected, slightly in the cervical region and with increasing intensity until the lumbar region is reached, where the changes are at a maximum. The sacral portion is less affected. The crowding of the perivascular spaces with cells is much less prominent than in encephalitis or poliomyelitis; but the characteristic diffuse infiltrations, spreading out

from the perivascular spaces, and diminishing as the distance from the vessels increases, are frequent and give the key to the histological diagnosis.

Turnbull and McIntosh, in discussing the distinction between this condition, poliomyelitis, and lethargic encephalitis, state that its resemblance to the former is in distribution rather than in histological detail, the lesions being less destructive and inflammatory in nature than they are in poliomyelitis. On the other hand, it resembles encephalitis more in histological details than in distribution, the altered areas in vaccinal encephalitis being more restricted in distribution and affecting, in a majority of cases, mainly the brain stem and the substantia nigra.

Apart from histological considerations, the causal rôle of the vaccinia virus in these cases is supported by the similarity of the changes found to those observed in the brain in small-pox with cerebral complications; by the similarity of the lesions found in the other organs in fatal cases of this disease to those experimentally producible by the vaccinia virus; and, finally, by the demonstration of active vaccine-virus in the brains of certain of the fatal cases.

In one case of post-vaccinal encephalitis McIntosh and Scaiff (1928) described multiple milary foci in the lungs, closely resembling milary tubercles, no bacilli could be demonstrated, and on more critical examination they concluded that they were dealing with granulomatous lesions. The same type of nodule can be produced by the intravenous injection of vaccinia virus into rabbits, and they have been figured in the lung by Levaditi and Nicolau in their work upon vaccinia virus. Similar granulomatous nodules have also been observed by other workers in the tissues of experimental animals whose appearance definitely recalls those met with in the post-vaccinal encephalitic cases.

It seems clearly proven that in certain cases the vaccinia virus may undergo generalisation, and by implication of the central nervous system produce untoward results. To what extent this is a new phenomenon is not at all clear; but it would seem certain that the virus must become widely diffused in every case of vaccination and not merely remain local. It is conceivable that a

strain of vaccinia virus might develop a special neurotropic character, but the experiments carried out by McIntosh, in the cases of vaccinal encephalitis referred to, do not lend any support to this view. It was a finding of Levaditi's that the neurovaccine developed by him adapted itself to the rabbit's brain in a way comparable to the adaptation of the rabies virus in becoming *virus fixe*. There would therefore seem, on theoretical grounds at all events, some danger in using such a virus for human vaccination. However, no nervous complications have been recorded in the small series of cases vaccinated with the neurovaccine, and the rather unexpected result of lack of virulence for the monkey on intracranial inoculation was obtained. The question of the cause of such determination of the virus to the central nervous system, as has occurred in these fortunately rare cases, whilst a matter of the highest practical importance remains for the present wrapped in mystery.

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## THE INFECTIVE THEORY OF MALIGNANT DISEASE

There is an undeniably great attraction about the idea of an infective origin for cancer. Though such an origin intuitively springs into the bacteriologist's mind, it cannot be denied that at the present time the matter bears the appearance of the wish begetting the thought. The problem of malignant disease is so pressing that one turns hopefully to this process, in the workings of which most of the advances of the last century in our better comprehension of disease have been made, for promise of enlightenment. Infection, if it were proven, would be an immense step forward ; it would immediately define the problem and open the way for the prevention and treatment of the disease. Yet, in spite of the enormous amount of attention given to it, the wish is far from being realised.

It certainly appeared, some three years ago when the work of Gye and Barnard was announced, that evidence of this nature was at last definitely forthcoming. The time that has passed since has brought no further progress upon these lines from which so much was hoped, and opinion at the present time is definitely moving away from the conclusions of those authors. This work, which nevertheless represents the most serious contribution towards an infective theory of cancer which has yet appeared, is worthy of careful review, since it has received very widespread publicity.

It has been an article of faith with most workers upon experimental tumours that the race of tumours is immortal, given sufficient and suitable pabulum for the continued growth and multiplication of their cells. By this it is to be understood that when a tumour is transplanted from one animal to another, and kept going year after year, the tumour cells are throughout the progeny of those of the initial tumour. The body of the host is merely so much culture medium from the point of view of the tumour cells. When a tumour " X " starts in Mouse No. 1, and

is transferred by repeated inoculations until it is growing in Mouse No. 100, its cells are still the direct descendants of those which originally assumed malignant growth in No. 1. The subsequent mice merely serve to nourish and perpetuate the parent growth which, if our technical resources were sufficient, might be propagated all this time in simple tissue culture. It is the general belief of pathologists that in these transplants a new tumour does not originate in the host animal; it is the old tumour which goes on growing. In this respect the tumour cell behaves like a staphylococcus or any other simple bacterial parasite.

For this reason transplantation experiments, although they have their uses, do not go to the core of the present question, which is: What is the cause of the original disturbance of growth which constitutes a neoplasm?

A very striking exception to these commonplace experiences, which we have perhaps laboured a little, was discovered by Peyton Rous in 1910, when he chanced upon the fowl tumour now widely known as the Rous tumour, or Rous sarcoma. This growth occurred in a Plymouth Rock hen, and was originally described by Rous as a spindle-celled sarcoma. The growth was transmissible to other fowls, and at first showed a remarkable limitation in this respect, only being capable of propagation at the outset in animals which were not merely of the same breed as the original fowl, but were actually related to it. All attempts to transplant the tumour into Plymouth Rock fowls of other stocks failed. Gradually this high degree of specificity became lost, and the tumour developed the ability to grow in other breeds until successful inoculations became the rule with fowls of all types. The growths behaved as highly virulent sarcomata, and became generalised, the animals dying with metastatic deposits, especially in the lungs. Now Rous made the startling discovery that this tumour, unlike all previously known tumours, was transmissible by means of cell-free filtrates (V and N Berkefeld filters). It therefore appeared indubitable that in each successful transmission by such means, the growth must originate *de novo* in the host's tissues, these being stimulated into neoplastic activity by a filtrable agent in the inoculum.

This phenomenon was a new one in our knowledge of the production of malignant tumours, and differed fundamentally from all other known methods of initiating malignant disease, which involve, practically without exception, the long-continued application of irritant substances to the tissues.

The growths, in the case of the Rous tumours, appear rapidly and after a single inoculation with a minute quantity of the infective material. It is, indeed, in many cases sufficient merely to insert a needle into a tumour mass and then plunge it into the breast muscles of a fresh fowl to cause the production of a growth.

These properties of the growth have remained unique. The transmission of other tumours by cell-free filtrates, and even a linkage of their characteristic properties with those of the Rous tumour, were wanting until the results put forward by Gye were published. The Rous tumour is undoubtedly conveyed by something in the nature of a virus, that is to say, by a body of simpler structure and smaller size than the animal cell; a body which is capable of passing through filters which hold the latter back and which, in this instance, displays properties of resistance to physical agencies which in certain respects are far greater than those possessed by animal cells. Since the serial transmission of the growth, *ad infinitum*, is a possibility, it follows that the infecting substance must be renewed in the course of this process. These criteria are those applicable to many other filtrable viruses.

Since the original tumour of this type was described by Rous, other observers have met with growths in the fowl whose biological properties are similar to those of the Rous growth, although their histological characters may be different, *e.g.*, Tytler (1918), Rous and Murphy (1914), Begg (1927), McGowan (1928).

The unique character of these growths raises two questions.—

(1) What is the position of these tumours amongst the neoplasms?

(2) What is the nature of the infecting agent?

Rous described the tumour which he discovered as a sarcoma. It is evident from the special characters of this growth, and the bearing which it has upon the infective theory of tumours generally, that any question of its not being a true sarcoma is of vital

importance. The fact has been challenged in this country by Leitch (1927), who doubts its true sarcomatous nature, and therefore regards all the work done upon this and similar growths as having no direct bearing upon the cancer problem at all. It is evident that such tumours are not uncommon in fowls, but as far as we know at the present time they are restricted to this species. Their general characters, progressive growth, invasive qualities and metastatic secondary growths, are more closely paralleled by malignant tumours than by any other known condition. If this is not to be called a malignant tumour, it may well be asked, why is it not so? And what then is it? The answer given by those who oppose this view to the first of these questions appears to be. "Because it is transmissible by a cell-free filtrate, which no other tumour is." This attitude is again countered by the believers in the truly sarcomatous nature of the growth as being a mere begging of the question, whose logical pursuit would lead to a progressive narrowing down of the list of tumours every time a distinctive property were discovered in any one of them. It may well be held that to add to our familiar definition of a tumour the further phrase, "not due to a filtrable virus," would be to make an entirely unjustified assertion; but at the same time we cannot lightly dismiss the important ætiological difference which distinguishes the Rous type of fowl-tumour from the great bulk of malignant growths as they exist in other species. A fact to be recalled in this connection, which may prove to be of much significance, is that an experimental sarcoma of the fowl induced by Murphy and Landsteiner by the injection of tar and embryonic tissue, proved transmissible in series, but that all attempts to propagate it by cell-free filtrates have given consistently negative results.

Leitch, striving for a definition of the Rous growth, refers to it as a "spreading necrobiosis," and lays much stress upon its highly necrotic character and the small amount of living tissue found in comparison to the large mass of necrotic material. It is doubtful if this is really a very valid distinction, since Rous has stated that the necrotic character of the growth, as now seen, was not an original characteristic, but appeared along with the assump-

tion of an increased degree of virulence. Carrel, who has studied the properties of this growth with great minuteness, always refers to it as a sarcoma, and it is evident from his writings that he freely accepts it as such and its properties as having a direct bearing upon the sarcoma problem in general. It may be said that whilst the opponents of the sarcomatous nature of these fowl-tumours are in a minority, and are sustaining a position difficult of defence but easy of attack, the remarkable and outstanding infective characters of the Rous tumour must make the question of its identity with the sarcoma series an open matter, unless and until some definite linkage with the latter is forthcoming.

Our knowledge of the properties, if not of the nature, of the infective agent have been advanced most largely by Carrel, who has studied it in tissue culture, in all phases of which work he is to be regarded as a master. Carrel found that in cultures from Rous' original sarcoma three varieties of cell grew out from the implanted fragment: polymorphonuclear leucocytes; macrophages (monocytes); and fibroblasts. In pure cultures the virulent agent was found to be associated almost exclusively with the macrophages, and cultures of these cells from healthy sources, when contaminated with virulent filtrates from the Rous sarcoma, became themselves infective, transmitted the infection from generation to generation, and reproduced the virus. In this way an active virus might be perpetuated in cell cultures. In the case of the fibroblasts the virus tended to disappear, and although detectable in the cells themselves, could not be found in the circumambient fluid, in which, in the case of the macrophage cultures, it was present in large amounts. In many cases, though not in all, the monocytes with which the Rous virus was associated showed definite morphological changes, assuming a fibroblast-like form and dying out with greater rapidity than is normal, although proliferation actively continued. Carrel assumes that the Rous growth is essentially a disease of the macrophages, which is brought about by the action of the virus. The effect on the cells is to cause their premature decease and break-up, with liberation of fresh quantities of virus. He explains the unlimited growth of the sarcoma cells, *in vivo*, as being due to the fact that macrophage cells belong to a type

capable of growth in serum which, according to his views, both lacks the essential substances usually requisite for cell-growth and normally exerts an inhibitory effect upon this. In the case of the diseased macrophages the necessary growth-promoting substances are liberated by their breakdown.

It is not quite clear if Carrel regards the Rous virus as a *contagium vivum* or not, but he appears to tend towards the latter views and look upon it, although he does not say so, in much the same way as a majority of bacteriologists regard the bacteriophage, viz, as an agent which sets up a disease process in the living cell which in its working sets free fresh quantities of the same disease-producing material. In fact, Bordet's phrase of an "hereditary nutritional vitiation" might well be used to describe the results obtained by Carrel with his diseased monocytic cells. In certain other experiments Carrel found that the ordinary, limited, and strictly determined proliferation of embryonic tissues, which as has long been known, takes place when these are inoculated into the body of an animal, might, in the case of fowls, be changed into a true malignant growth if such substances as indole, tar, or arsenious acid were introduced in small quantities at the same time as the embryonic tissue. These results, obtained presumably under strictly aseptic conditions, seem to indicate a physical agency as the prime mover in the initiation of the process rather than anything of the nature of a *contagium vivum*, at any rate of external origin.

As is the case with the bacteriophage, the production of the virus depends upon the active multiplication of living cells, and it undergoes no increase in the absence of this. In serum-broth or in saline solutions it is highly labile, as Rous found long ago, and either disappears or loses its activity. In the presence of dead tissues it may survive for some days, but ultimately dies out here too.

The gap between these fowl-tumours with their special characters and the human tumours, or the rat and mouse tumours which so closely simulate them, seemed to have been bridged by the work of Gye and Barnard (1925) and the wide divergencies composed under a single broad conception. Gye's work turns upon a

crucial experiment in which the Rous virus is split into two portions, neither of which is active alone, but which together reproduce the properties of the original virus. The effect may be roughly likened to the action of complement and hæmolysin upon red cells. These two constituents of the virus, according to this view, are of the nature of a cultivable microorganism and a specific chemical fraction which activates the organismal one. The further and highly important result was claimed, that the organismal factor might be substituted by an identical organism cultivated from human or other non-filtrable cancers, thus linking up the Rous virus with the non-filtrable tumours.

These conclusions were reached by varied experiments, of which we may quote certain examples. Gye found that portions of a Rous tumour, placed in broth, communicated the infective factor to this broth. These infective broths he regarded as cultures; although simple diffusion of the virus would suffice to account for the phenomenon. He found, as has already been mentioned, that the infectivity of these "cultures" fell off and finally disappeared as time passed. Let us call such an inactive preparation "A." Turning to another type of experiment, he found that saline extracts of disintegrated tumour tissue, which were of course infective, might be rendered inert by careful treatment with chloroform. Let us call this preparation "B." Now Gye found that the two substances thus prepared, "A" and "B," inert when injected separately, would when acting together set up a Rous sarcoma.

The same result was obtained by centrifugalisation, although with a certain lack of sharpness. By this procedure, carried out with "cultures," the infectivity both of the deposit and of the supernatant fluid when acting alone was found to be *nil*, or very slight. When the two were mixed the material was once again definitely infective.

The interpretation placed upon these results, by Gye, is that in such materials a living virus is present, which is capable of cultivation, the virus alone is incapable of initiating tumour-growth, but when associated with a non-particulate, soluble factor, it becomes fully active.

To this point the case of the Rous sarcoma alone, a suspect tumour as we have previously indicated, has occupied our consideration. The more revolutionary findings may now be mentioned. Gye found that the virus portion of the tumour-producing complex could be obtained in cultures, according to his technique, not only from the fowl-tumour itself, but also from the mouse sarcoma "87/s" of the Imperial Cancer Research Fund; from Jensen's rat sarcoma; from a carcinoma of the mouse, "strain 68," and a rat carcinoma, "strain 9," both of the Imperial Cancer Research Fund; and, in addition, from a human adenocarcinoma of the breast. He could thus produce the Rous sarcoma with factor "A" from very varied malignant growths, and factor "B" from the Rous tumour.

Gye consequently enlarged the interpretation, which he applied to the apparently dual nature of the infecting agent, to conceive of the cultivable virus as being a single common agent of all malignant tumours. The specificity of these, and the determination of histological type, he believes to lie in the specific nature of the soluble factor "B". On this assumption he explains why in every case the result of his experiments was the production of a Rous sarcoma. He believes that the malignant tumour virus is a widely spread material in nature, capable of invading any host, but that its activity, the site of this and the nature of the growth resulting, waits upon the presence of the activating specific factor.

A point in this work, which we have not as yet touched upon, is the matter of the serial propagation of the virus. As described above the cultural experiments would be unconvincing, since simple diffusion of the active agent, plus the effects of dilution, would suffice to explain all of the results achieved. By making sub-cultures from his initial diffusions, or cultures, into media containing chicken embryo, Gye succeeded in obtaining positive results, as regards the cultivable factor, with such sub-cultures of the Rous virus in the sixth and eighth generations. It is not perfectly clear if these were obtained with this tumour only, or with the rat, mouse, or human growths as well.

These results, if correct, would obviously have an enormously

important bearing upon the whole of the cancer problem and necessitate a complete reorientation of our ideas with regard to it. The attention of numerous cancer workers was immediately focussed upon them and the work, and the views which it implies, have received a thorough examination at their hands. It would appear that if Gye's hypothesis were correct it should be possible to reverse the experiment, and to obtain other of the experimental series of tumours, besides the Rous growth, by interchange of virus and specific factor. This has not been done, and so far as we can ascertain, *the experimental result throughout this work has always been the production of the Rous sarcoma*. Now the fact has been reiterated by Carrel, Leitch, and others that the fowl is almost infinitely variable in its susceptibility to the Rous virus, and Carrel has stated that it is an impossibility to compare an experiment made upon one bird with an experiment made upon another, even under identical circumstances, on account of this individual variation. He himself devised a method for the comparative examination of the activity of different Rous preparations, consisting in the insertion of small discs of flannel, soaked in the virus, into different parts of the breast of the same bird. By this means he states that a rough comparison of the activity of different dilutions of the virus can be made. In the case of many of Gye's experiments it seems probable, as Leitch has suggested, that the "inactivation" of the virus consisted simply in its attenuation to a degree at which the chances of its giving a positive result were low. Such a result might then occur in certain cases owing to individual variations in the animals, or be produced by the intervention of other factors not of a specific nature. Leitch has stated that in certain experiments the so-called chemical factor alone gave rise to tumours, and that in others the combination of the two supposedly essential factors was found to be ineffective.

In considering the possibility of the activation of filtrates and extracts of the growth, whose activity has almost been extinguished by chemical means such as chloroform, we may recall the results of Carrel, who has shown that the action of the Rous sarcoma virus is intensified by the presence of non-specific substances in great variety, and has established the fact that a very

small amount of the Rous principle, which fails to produce any effect when injected alone, will determine the presence of a tumour if the tissues are slightly irritated. One factor in bringing about a positive result in such borderline experiments may be the determination of macrophages to the site of injection, these being cells peculiarly susceptible to the Rous virus and the type especially favourable for its increase. In Gye's experience extracts of the Rous tumour, when inactivated by chemicals, could only be reactivated by extracts of other tumours, normal tissues and embryonic tissues having no such effect. Murphy (1926), however, states that he has been able to reactivate chloroformed extracts of the Rous sarcoma by "cultures" of chicken embryo and rat placenta; he inclines to the view that the virus in such experiments is attenuated rather than inactivated, since an excess of chloroform results in its being irrevocably damaged. Mackenzie and Illingworth (1926) were unable to substantiate Murphy's results, but they were also unable to obtain any activation of chloroformed filtrates by "sub-cultures" of the Rous virus. Baker (1926) obtained results which to some extent conform to those of Gye, in that he was able to activate chloroformed extracts of the Rous tumour by the addition of "primary cultures" (? diffusions) of pieces of Jensen's rat sarcoma. In one case a similar result was obtained with a "secondary culture." Baker points out, as is abundantly evident from the details of his experiments, that such an activation showed itself by a quantitative effect rather than by clean differences between experiment and control. In many of his animals the supposedly inactive filtrate gave rise to tumours, which however were definitely smaller than those in which the activating "culture" was present. The further and most significant observation was made that the same process of reactivation could be observed in preparations of the Rous virus which were merely diluted towards the point of extinction of their activity, and in which no question of any differential action of chloroform upon a constituent of the virus was involved.

Kolmer and Harkins (1927), who have carefully repeated much of Gye's work, conclude that when once the Rous tumour extracts

are treated by chemicals, or by heating, to a point at which their inactivation is certain, then their infectivity is entirely and permanently destroyed. They failed to find any evidence of a "specific factor," which they believe to be "nothing more than a suspension of attenuated living virus which may or may not produce a tumour depending upon the fowl's susceptibility or resistance." To this we would add the further conclusion, from the results of other workers, that the last-named factor is probably capable of local alteration by the results of the injection, the exact tissues encountered at the site of deposit of the inoculum, and by the non-specific materials contained in it.

It will be seen, from what has been said, that in the period which has elapsed since this work was originally brought forward no unequivocal confirmation of it has been forthcoming, although it has aroused an immense amount of interest and is referred to in every current journal upon the cancer problem. The seemingly inevitable conclusion, from the available evidence, is that the proposition is untenable or at least unproven.

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## CHAPTER IX

### ULTRAMICROSCOPIC AND FILTER-PASSING VIRUSES (*contd.*)

#### THE INFLUENZA PROBLEM

PFEIFFER discovered the Influenza Bacillus in 1892. For about twenty-five years this organism was the accepted cause of the disease, but coincident with the fall of the Habsburgs and the Hohenzollerns the position of the influenza bacillus became badly shaken. After the decline of the 1889 epidemic, influenza in this form disappeared from our ken until the terrible outbreak which occurred in the last years of the war. In the interval we were acquainted only with what was called "influenza" in a sporadic form and in small and localised outbreaks, and we have no certain knowledge by which to identify these occasional illnesses with epidemic influenza, of which our only really definite criterion is its epidemic incidence. Until the influenza organism is known and identified we cannot say what is influenza, and what is something that merely clinically simulates it.

The 1918-19 epidemic was first seen by the writer amongst British troops in Italy in the summer of 1918. The coming of the disease, "Spanish influenza," as it was called at the time, had been heralded, and when it arrived its incidence in every unit was extremely high. The disease was an acute febrile one, without localising signs and without mortality. In the hundreds of cases seen by the writer no fatal case was observed during the first onrush of the disease. Prostration was great, but recovery was rapid, and by the time cases had reached casualty clearing stations, had been through the business of admission and were seen by the medical staff, the majority were apyrexial. So unlike the then prevalent conception of influenza was this "three-day fever" that the opinion was very widely held that a new disease had arisen, much as trench fever had arisen at an earlier date.

The first wave of the infection passed, and with decreasing incidence the severity of the symptoms increased, the duration of the illness lengthened and, above all, pulmonary complications appeared. How much the absence of these latter during the earlier phase of the disease was attributable to the mildness of the Italian summer, which was now replaced by a raw and frozen winter, or how much their presence was due to the natural evolution of the epidemic, apart from climatic conditions, it is impossible to say. The unfortunate thing is that during the early and uncomplicated phase of the disease, on account of its unlikeness to the then current conception of influenza as seen in non-epidemic times, Pfeiffer's bacillus was very rarely searched for upon a large scale, and the opportunity was missed of settling once and for all whether it was present in the uncomplicated disease or not. In other parts of Europe, and in America, very contradictory reports were made, but the general impression gathered from them is that the examination for the bacillus was rarely carried out upon a large scale and with efficient technique in these earliest days of the pandemic, and, secondly, that those who made extended examinations did not find the organisms as frequently at this stage as in the later period, when complications were more prevalent. Kristensen, in Copenhagen, examined the nasopharynx in 135 soldiers suffering from the uncomplicated form of the disease, in July, 1918, and only found Pfeiffer's bacillus in nine instances, which, as the author's results show, is no more than might be found in healthy subjects. Ledingham, who observed the disease in Mesopotamia, states that he was unable to find it in these cases, but unfortunately no other wide investigations of this point seem to have been made, with the exception of those of Blake, Rivers and Small, which, although made upon "uncomplicated" cases of influenza, were carried out at a time and in an area in which numerous fatal cases of the disease were current. These workers found Pfeiffer's bacillus present in almost all of their cases.

The question of the presence or absence of this organism in the earliest stages of the uncomplicated disease is, of course, a vital one from the point of view of its claims. One must admit at

once that had the idea of making a systematic search for the bacillus occurred in the summer of 1918 the worker would have been at a loss to know where to look for it. Naturally, and traditionally, the throat and nasopharynx would have been examined, but only in a real minority of cases were any throat symptoms complained of, and it seems difficult to conceive how so sudden and prostrating a disease could be occasioned by bacilli of no very high pathogenic powers without the presence of some local symptoms.

The influenza of the later stages of the epidemic was a very different matter. In the cases which ended fatally a complicating broncho-pneumonia was present in which the bacterial flora was mixed; Pfeiffer's bacillus was commonly encountered, especially in the early stages, but the organisms chiefly implicated in the diseased lungs were the common mouth varieties of pneumococci, and hæmolytic streptococci, as the studies of Opie, Blake, Small and Rivers have clearly shown. Moreover, it had become evident by this time that the epidemic in question was one of real influenza. Attention had been turned to the search for Pfeiffer's bacillus, and most laboratory workers had developed a satisfactory technique for its isolation. In the earlier days of the epidemic the medium most widely used was simple blood-agar, and it took some time for bacteriologists, many of whom were rather unfamiliar with the hæmophilic group, to discover the unsuitability of this medium and the selective character of boiled blood-agar, Levinthal's agar, etc.

Pfeiffer's bacillus was abundantly present, often in almost pure culture, in the sputum from the cases of influenza with bacterial pulmonary complications. The organism, moreover, although not of very high pathogenicity, has quite definite pathogenic abilities. In large doses it kills rabbits with rapidity, both by intravenous and intraperitoneal injection, and when given by the latter route brings this about without any evidence of leucocytic reaction, either local or general, and with the presence of hæmorrhages and ecchymoses in various parts of the body, but especially in the suprarenal capsules. The organism is therefore pathogenic for laboratory animals, and Parker has shown that a fairly potent

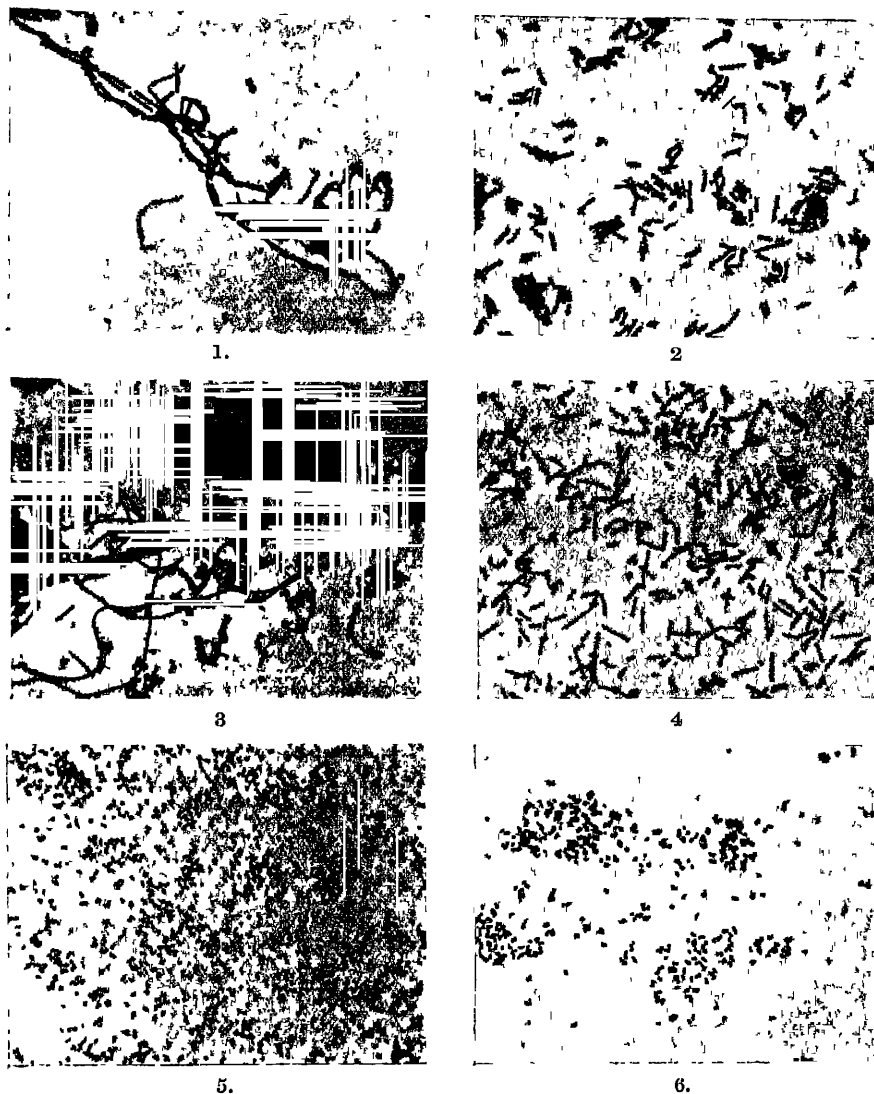


FIG. 12.—1, 2, Hæmolytic hæmophilic bacteria from the throat,  
3, 4, Large non-hæmolytic hæmophilic forms (Type "B," Dible),  
5, 6, True Pfeiffer bacilli ( $\times 850$ ).

toxin is produced in young broth cultures. This, too, in sufficient dosage will kill rabbits, but both in the case of the toxin, and with cultures of the organism, relatively large doses are required to demonstrate these effects, and it cannot be said that the picture of human influenza has been reproduced with any fidelity. In fact, one of the great difficulties in the way of the investigation of this disease seems to be the lack of an animal in which it can be faithfully reproduced.

**Bacteriology of the Pfeiffer Group.**—There are no very strict criteria as to what constitutes the influenza bacillus. Its small size and strictly hæmophilic characters have long been its accepted attributes, but further investigations by Pritchett, Stillman and Bourn (1919–20), the author (1922), and Kristensen (1922) have shown that the hæmophilic organisms of the respiratory passages of man form a very heterogenous group and include —

- (1) Small, hæmophilic, non-hæmolytic bacilli (Type Pfeiffer)
- (2) Large, often filamentous, ditto (Type “B”; Dible).
- (3) Hæmolytic hæmophilic bacteria (Type “X”, Pritchett and Stillman).

The first of the above groups is the one usually regarded as the true Pfeiffer bacillus, and the third group is generally looked upon as non-pathogenic; quite why is not clear, and the matter still remains open for investigation. The organisms in the second group are only doubtfully members of the Pfeiffer group, and are distinguished by morphological differences, often extremely marked (Fig. 12). Into this class may fall many of the organisms causal of what was formerly called “acute leptothrix meningitis,” which have been found by Henry (1912) and others to be hæmophilic in their growth requirements. The number of cases of meningitis due to hæmophilic organisms is not inconsiderable. Many of these are occasioned by organisms frankly of the Pfeiffer type, but in others the bacilli form long thread-like filaments (Mackenzie, 1927). Henry found that in certain instances the leptothricoid morphology was a function of age and cultural conditions. This is certainly not always the case, since, in the saprophytic types of this nature studied by the author in the normal

throat, the morphology was strictly characteristic and invariable. In the view of some workers these atypical organisms should be included in the true Pfeiffer group, although Pfeiffer himself established a pseudo-influenza group for organisms of aberrant morphology. Whilst agreeing that there may sometimes be difficulty in making a morphological distinction between very similar forms, it would be inexcusable, if morphology means anything in bacterial classification, to include some of the long-thread-like types encountered, with the rather sharply defined, minute cocco-bacilli of Pfeiffer. Furthermore, many of the organisms of this type, isolated by the author, fermented saccharose which was never attacked by the true influenza bacilli, and none of them formed indole, which about 40 per cent. of Pfeiffer strains do.

Within the Pfeiffer group itself, as here set out, numerous subtypes may be distinguished by sugar reactions and other biological tests. The group can be split into two large classes on the basis of the indole test and into further subdivisions by fermentative reactions. The present view is to regard these subdivisions as of no importance, but it must be admitted that there are no very good experimental reasons for this attitude. Immunological methods have not yielded any further information, since the types have been found to be multiple. It may, however, be of some significance that certain studies upon the hæmophilic bacilli of meningitis show that these strains are far less diversified than are those isolated from sources open to contamination (Rivers and Kohn, 1921). It may well be possible that the pathogenic faculty is restricted to only a small proportion of the large population of influenza bacilli normally living upon the human subject.

**Growth Requirements of *B. Influenzæ*.**—It has been stated, from respectable antiquity, that the influenza bacillus would only grow in the presence of blood or hæmoglobin, but in the pandemic of 1918–19 most bacteriologists soon discovered that plain blood-agar was an unsuitable medium for this bacillus and that growth upon it only took place satisfactorily in the vicinity of colonies of some other organisms. It was also commonly found that heating the blood increased the effectiveness of the medium; that a few minutes' exposure of the blood-agar to a temperature of about

90° C. produced an optimum effect, which was not much decreased by boiling the mixture for as long as fifteen minutes. Others found that destruction of the blood by digestion or by chemicals similarly enhanced its action.

The study of the growth requirements of the bacillus was taken up in more detail by Davis, and by Thjotta and Avery, who showed that neither blood, nor blood derivatives, were the essential factor, but that cultivation of the organisms might be achieved in ordinary broth to which traces of extracts of bacteria, yeasts or fresh vegetables had been added. The nutritional effect of such extracts was negligible, so that their action resembled that of one of the accessory food factors so well known in mammalian physiology. This stimulating substance was thermostable; resisting boiling for ten minutes, but being destroyed by autoclaving. On account of its similarity to a vitamin, Thjotta and Avery designated this growth substance the "V" factor. It was found that although growth took place readily in media containing this factor, cultures could not be carried on in continuous series. Some further substance was lacking which was present in the earlier growths (transferred from blood media) and was presumably carried over from them in sufficient amount to support growth for a limited period. This is referred to as the "X" factor, and was suspected in the first place of being a hæmoglobin derivative. The explanation here suggested to explain the continued growth in media containing only the "V" factor was shown to be correct by the complete inability which the organisms displayed, when washed free of their original culture medium, to show any growth when transplanted to the medium containing "V" only. Thjotta and Avery found that the quantity of the "X" factor requisite for growth was extremely minute, so small as to suggest that it acted as a catalyst. This substance could be separated from the "V" substance by its greater resistance to heating. It is not destroyed by autoclaving at 120° C for thirty minutes, which completely abolishes the "V" factor. Blood was found to owe its special favouring effect to the presence of both "V" and "X" factors, which reside mainly in the corpuscular elements. As an experimental source of the "V" factor alone,

boiled extracts of yeast were found to be most suitable. The matter may be summed up as follows :

(1) Boiled blood (V + X)	=	Favourable for growth
(2) Autoclaved blood (X)	=	Unsuitable „
(3) Boiled yeast extract (V)	=	Unsuitable „
(4) Autoclaved blood + boiled yeast extract (V + X)	=	Favourable „

The yeast extract ("V" factor) thus reactivates the blood medium which has been inactivated by excessive heating.

The "X" factor is definitely related to the hæmoglobin element of the blood, and is abundant in crystallised hæmoglobin which has no oxygen-carrying capacity. It has not been possible to replace hæmoglobin derivatives by any simple iron salts and, on the other hand, the iron-free hæmoglobin derivatives, such as hæmatoporphyrin, are without effect (Davis). The beneficial effect of altered blood on the hæmophilic organisms, as distinct from the unaltered corpuscles of ordinary blood-agar, is probably due to a setting free of the "V" factor in the breaking down of the red cells. This is supported by the fact that mere traces of the "X" substance are necessary, which would be present in unaltered or slightly altered blood, whilst much greater concentration of the "V" substance is required. That the effect of boiling the blood is not due to liberation of the "X" substance, or to mere destruction of active hæmoglobin, is shown by the fact that its prolongation leads to a progressive diminution in the specific effect, a procedure which has no appreciable action on the highly thermostable "X" substance in the concentration in which it is present in such media.

Thjotta and Avery have further shown that blood, or blood derivatives, are not essential for the maintenance of bacteria of this group, and that both "V" and "X" substances are present in fresh vegetable tissues such as potato. They state that Pfeiffer's bacillus will grow upon Uschinsky's synthetic medium in the presence of sterile raw potato. The plant tissues, as is well known, contain catalase and oxidases, and the "X" factor, which in blood is so definitely related to the latter, probably

resides in the same factor in the plant tissues. The general conclusion seems to be that the "X" substance is a catalyst, and perhaps an oxidase, whilst the "V" substance is more complex organically and a growth product of tissues and bacteria. There is one difficulty in the way of this concept which rests upon an isolated experiment by Olsen (quoted by Kristensen, p. 117). This worker found that whereas crystalline hæmoglobin alone was useless as a growth-promoting factor when added to agar, full activity was obtained when the agar containing it was boiled. In other words, the crystallised hæmoglobin behaved exactly like fresh blood. If this observation is correct it points to a very much simpler composition for the "V" substance than the bulk of experiments upon it have suggested. Before leaving this subject it may be noted that Fildes (1924) found that two of the other organisms of the hæmophilic group differed from Pfeiffer's bacillus in their growth requirements. *B. hæmoglobinophilus canis* is capable of continued growth in a broth containing the "X" factor alone, and devoid of the "V" factor; it therefore, except for its dependence upon the "X" substance, behaves like yeast and other non-hæmophilic bacteria in that in its growth it synthesises or sets free the "V" factor. Fildes showed that it might even be used as a source of the "V" factor in the cultivation of other hæmophilic organisms. He also found a converse state of affairs in the case of the hæmophilic hæmolytic bacilli. These were able to be propagated for a number of generations in media containing the "V" factor only. The suggestion made was that the organism itself was capable of supplying the "X" factor.

**Distribution of Pfeiffer's Bacillus.**—The organism is a normal inhabitant of the human throat. Gibson and the author found it present in 80 per cent. of healthy medical students examined on a single occasion in a non-epidemic period, and others have had similar experiences. There is, however, a strong probability that the incidence of the organisms undergoes marked seasonal fluctuations and variations from other causes. The bacillus has been repeatedly found in the throat and sputum, in as many as 50 per cent. of patients with measles, tuberculosis, bronchopneumonia, lobar pneumonia, etc.

**The organism and influenza.**—If we examine this question in the terms of Koch's postulates, we must begin by admitting that we cannot confidently assert that the organism is present in every case of the disease. We have emphasised the doubt about its presence in the uncomplicated forms of influenza, although it is certainly present in a high proportion of cases with pulmonary complications and in numbers which are sometimes very striking, films of the sputum often being crowded with Pfeiffer's bacillus to the exclusion of other organisms.

The experimental investigation of the disease in animals is limited by the fact that laboratory animals are not susceptible to influenza and our criteria of infection are unsatisfactory. Pfeiffer's bacillus, or types of it, are undoubtedly pathogenic, and will cause very severe lesions in animals, but they do not produce a condition at all comparable to the disease influenza. With regard to collateral evidence, it has been found by a number of workers that agglutinins and complement-fixing antibodies for Pfeiffer's bacillus develop in the course of the disease: these are usually most marked for the homologous strain, as would be expected from the general serological heterogeneity of the group. The significance of this, however, is not greater than to point to the fact that the bacillus plays some part in the morbid process, a matter which is hardly in dispute.

There remain the attempts which have been made to solve the problem by direct experiments upon man and the anthropoids. The most weighty results are those of Blake and Cecil (1920), who utilised a strain of influenza bacilli whose virulence had been assured by passage through mice and monkeys. This procedure has been much criticised, but to us it seems that in the absence of any means of distinguishing virulent influenza bacilli from the presumably saprophytic inhabitants of the normal throat, the use of a fully virulent strain was right.

Twelve monkeys had quantities of culture instilled into the nose and mouth, and all of these came down, after three or four hours, with an illness, ushered in by marked prostration and coryza, which lasted from three to five days, and was either recovered from or appeared to be going on to recovery when the animals were

killed. Cough developed in twenty-four to forty-eight hours, the temperature was sometimes elevated, but not markedly so, and leucocytosis was absent unless other infections complicated the condition. A clinically recognisable pneumonia developed in two cases. Ten monkeys were injected intratracheally with 1.0 to 5.0 c.c. of cultures of the same strain of Pfeiffer's bacillus, with results similar to those obtained in the previous series of experiments. In both series the *B. influenzae* was recovered from the lesions produced, either in pure culture or in association with the ordinary banal organisms of the upper respiratory tract.

These experiments show clearly that a strain of Pfeiffer's bacillus of sufficient virulence *can* cause a spreading inflammatory infection of the uninjured upper respiratory tract of monkeys, an infection which is accompanied by prostration and which shows a not inconsiderable similarity to the clinical picture of human influenza.

This is in some ways a rather remarkable result, for, as Cecil and Blake point out, the most virulent streptococci, organisms incomparably more dangerous to the animal than these influenza bacilli, have no such ability to set up naso-pharyngitis and tracheitis upon simple instillation in the way the Pfeiffer bacilli did, and the same has to be said even of the pneumococcus. These observers had previously successfully reproduced the whole picture of lobar pneumonia in monkeys by the intratracheal injection of virulent pneumococci, but their experiments failed when they limited themselves to simple instillation or to spraying.

In the altered lungs of the infected animals a picture not dissimilar to that seen in the bronchopneumonia of human influenza was discovered. Hæmorrhages, œdema and hæmorrhagic exudation with sparsity of fibrin, bronchiolitis, peribronchial infiltration, bronchopneumonia, bronchiectasis and emphysema, were demonstrated in many cases. The differences between these lesions and those in human fatal cases were those of degree, the changes being distinctly milder in the monkey. It is to be remembered, however, that a strict comparison from this point of

view cannot be made, since in the animals the disease produced was mild in form, and was going on to cure at the time they were killed for pathological examination. It may be noted that Cecil and Blake found the influenzal infection to spread down the bronchial tube and to involve the lung alveoli by contiguity from the finer bronchioles. The influenza bacilli, in contrast to pneumococci and streptococci, showed little tendency to invade the lymphatics or interstitial tissue of the lung, and appeared to damage the tissues by toxic action whilst growing on the surface of the respiratory passages and, in this sense, outside of the body.

On the other hand, a number of attempts to infect man with Pfeiffer's bacillus have only given a few definitely positive results. The experiments of Sellards and Sturm (1919), as well as those of Bloomfield (1920), may be discounted, since the former used strains of bacilli isolated from measles, and the latter those obtained from healthy throats. In both cases the organisms had been in culture for some time, and in both the results were negative. Certain negative results of other workers (Wahl, White and Lyall; M. J. Rosenau and McCoy and Richey) were not obtained upon a sufficient material to be of great value. Cecil and Steffen (1921) made a more extended and careful examination of the question upon the lines followed by the first-named worker and Blake in their monkey experiments. They used a culture isolated from the pleural fluid, in a fatal case of the disease, which was virulent for rabbits and mice. As a result of the nasal instillation and the swabbing of the throat with pure cultures, or with the peritoneal exudate from a monkey suffering from *B. influenzae* peritonitis, they succeeded in setting up in the majority of their volunteers a sharp illness, which began within a few hours of the inoculation and lasted from two to ten days, and was characterised by malaise, headache, sore-throat, cough, rhinitis and muscular pains. There was no leucocytosis and no pyrexia. The disease was in most cases mild and its evolution benign. Cecil and Steffen noted that the infection produced was milder than that seen in epidemic influenza, and they did not press the conclusion that the two conditions were identical.

### THE FILTER-PASSER THEORY OF INFLUENZA

The widespread dissatisfaction with Pfeiffer's bacillus as the causal agent of influenza directed attention towards the possibility of a filtrable virus being the real cause of the disease. The first positive findings of this nature were reported by Ch. Nicolle and Lebailly, in 1918. They inoculated the filtered sputum [Chamberland bougie L 12 (? L.2)] from cases of influenza subcutaneously and intravenously into volunteers, two of whom contracted an apparently typical attack of influenza after an incubation period of six days. De la Rivière (1918) stated that he had produced an attack of influenza in himself by similar means, and the theory of a filtrable virus also obtained considerable support from German workers, notably Kruse (1918), Selter (1918), and Leschke (1918), who all adduced similar experimental results. In Japan, Yamanouchi, Sakakami and Iwashima (1919) inoculated twelve persons with the mixed emulsified sputum from a large number of influenza cases, and a like number with Berkefeld (grade unstated) filtrates of the same material. The inoculations were made into the mouth and nose, and resulted in attacks of influenza in all the volunteers, save six who had previously had the disease. The incubation period was from two to three days. "A filtrate of blood" also proved infective for six further volunteers upon its injection into the nose and throat. Unfortunately the details of these experiments are too scanty for their ready acceptance at their face value.

The sum of these reported results, which were contradicted by other workers, is not very convincing, especially when it is remembered that all the experiments were carried out at a time in which influenza was epidemic, and how difficult it is under such circumstances to exclude satisfactorily the possibility of naturally occurring infection.

More weighty observations were those due to Wilson (1919); Gibson, Bowman and Connor (1919); and Olitsky and Gates (1921 *et seq.*), all of whom claimed to have solved the problem by the cultural method. Wilson, with Rose Bradford and Bashford, believed that organisms had been cultivated by the Noguchi

technique, not only from influenza but in a large number of other diseases, including trench fever, nephritis, encephalitis, etc. This work was speedily discredited through the demonstration by Arkwright of contaminations in the cultures submitted to him. The same worker also showed the presence of bodies identical with those assumed to be micro-organisms in control, uninoculated, cultures. These criticisms were in the main accepted by Wilson and Bradford. Gibson, Bowman and Connor, like the last-mentioned investigators, also worked in the British Army in France, and independently reached similar though less sweeping conclusions. They found that the inoculation of filtered and unfiltered sputum, taken at an early stage in the disease, produced lesions in the lungs in a high proportion of the inoculated animals: monkeys, rabbits, guinea pigs and mice. (The filters used were "Chamberland L. 1 bis" (? L. 1a) and Chamberland F.) These remarkable experimental results with the smaller laboratory animals are isolated, although Ch. Nicolle and Labailly had previously recounted the successful infection of monkeys with influenzal sputum. The cultivation experiments were carried out in Smith-Noguchi medium, and the presence of small coccoid bodies was observed in some cases. These were Gram-positive. This work, which makes rather confused reading, was interrupted by the death of Gibson from influenza, and by the upheavals of wartime research; it was left by its authors in a somewhat incomplete state.

The most serious contribution to this aspect of influenza is undoubtedly the work of Olitsky and Gates. These investigators in the first instance took up the study of the disease experimentally, employing the rabbit for this purpose. They proposed, as a criterion of infection, the production of a leucopænia involving essentially the mononuclear cells, which they believed to be a blood change characteristic of influenza. Their work divides itself into three portions:

- (a) The experimental results obtained in rabbits
  - (b) Cultural experiments.
  - (c) The immunological relationship of their virus to the disease.
- A. Using filtered (Berkefeld V and N filters) and unfiltered

naso-pharyngeal washings from early cases of influenza, they found that intratracheal injections of these substances produced definite pulmonary lesions in rabbits. These were of the nature of a hæmorrhagic œdema, with scattered patches of more definite hæmorrhage and emphysema. The condition developed in 24-28 hours, 'was associated with a fall in the mononuclear leucocytes; and the pathological changes were confined to the lungs, in which ordinary micro-organisms were either few or absent. Such a syndrome, which was regarded as experimental influenza, could be transmitted from rabbit to rabbit in series, by grinding up the damaged lungs, extracting with saline, and using this material as the inoculum for the next injection. The infective agent was found to persist after repeated passages had removed the ordinary sputal organisms present in the original inoculation material, in the cases in which this was used in the unfiltered state. The virus was readily filtrable (Berkefeld N) and resisted the action of 50 per cent. glycerol for long periods, although being rapidly destroyed by a temperature of 56° C. Before leaving the matter of animal experiments it may be noted that Olitsky and Gates failed to obtain the results in monkeys claimed by Nicolle and Lebailly, and also by Gibson, Bowman and Connor.

B. The second fact of importance in this series of observations was the cultivation of an organism from the naso-pharyngeal washings obtained in the early hours of uncomplicated influenza. By means of the familiar Smith-Noguchi medium they succeeded in growing a minute micro-organism, measuring about  $0.15 \times 0.8 \mu$ . These little cocco-bacillary bodies are not easily stained by ordinary dyes and are decolorised in Gram's method. They are non-motile and strictly anaerobic. Cultivation is difficult in the beginning, but the organism rapidly adapts itself to artificial media and gradually becomes less particular in its growth requirements, so that it is eventually possible to obtain surface colonies upon blood agar plates under anaerobic conditions. The colonies so formed are small, clear, circular and translucent, but the growth upon blood agar is always minimal. Gates (1922) has succeeded in obtaining mass cultures of the organism without the protein

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precipitates, which are a source of so much trouble and confusion in the Smith-Noguchi medium, by the device of placing the culture medium in collodion sacs, immersed in a jacket of sterile distilled water, the whole apparatus being anaerobic. Under these circumstances sufficient pabulum diffuses into the water to support a growth of the organisms. The authors have also found the bacterium capable of growth, under anaerobic conditions, in simple glucose broth in which a slight growth of *B. coli* has been permitted—this being killed by steaming as soon as it begins to cloud the medium.

The organism was named *Bacterium pneumosintes* by its discoverers, who were unable to decide as to its exact place within the existing bacterial groups. More recently the term *Dialster* has been coined by the Committee on Nomenclature of the Society of American Bacteriologists, to embrace organisms of this type (Bergey). Olitsky and Gates succeeded in obtaining cultures of the micro-organism from the nose and throat of a number of cases of influenza and regularly failed to find it in various other conditions, including acute coryza. It could be cultivated from the lungs of experimentally infected rabbits, as well as from human sources, and in pure culture was capable of producing the same pathological picture in rabbits as was produced by the nasopharyngeal washings of patients. The behaviour of *B. pneumosintes* being in all respects exactly the same as that of the unknown virus encountered in their earlier experiments, Olitsky and Gates were led to the conclusion that it was the microbic cause of influenza. They considered, both from general and experimental evidence, that it was the agent primarily damaging the lungs, and that the severe and septic lesions of influenzal pneumonia were secondary effects produced by non-specific organisms in a lung which was already laid open to invasion.

C. Having demonstrated this organism to their satisfaction in a comparatively large number of cases of influenza, Olitsky and Gates naturally proceeded to examine its immunity reactions in connection with cases of influenza. They were able, in the first place, to obtain the ordinary agglutination and fixation tests with inoculated rabbits, showing that the common bacterial attributes

were present in their organism, and they also demonstrated that the different strains isolated formed a single immunological group. Agglutination, nevertheless, appears to have been somewhat unsatisfactory, as even under the favourable conditions of artificial immunisation they were disposed to accept a titre of 1 in 8 as diagnostic. With regard to the more important question of antibodies in the sera of patients, agglutinins were detected in dilutions of 1 in 10 to 1 in 50. To obtain even these low titres the authors had recourse to the technique devised by Northrop and de Kruif (1921-22), in which the antigen is suspended in a phosphate mixture having a pH of 6.8, which was found to constitute an optimum condition for the reaction. The blood of a number of control subjects also gave agglutination in approximately the same titres, but, as the authors point out, in the case of a disease like influenza, unexceptionable controls are not easy to find.

Attempts were also made to carry out preventive inoculation experiments in man. As a result of inoculations with *B. pneumosintes*, which of themselves were practically innocuous, a low titre of agglutinins was developed, but decisive results in the way of protection were not obtained owing to the conditions at the time not favouring a sufficiently large scale experiment.

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The above summary puts forward the main facts elucidated by these authors, of whom it should be said that they have refrained from drawing the sweeping conclusions as to the importance of *B. pneumosintes* in influenza, to which their work might have tempted them. It remains now to review it and to consider, at the same time, the results obtained by others who have pursued the same lines of inquiry.

The results obtained in the rabbit have been to some extent confirmed but more generally disputed. A settlement of the question has not been reached, since the pandemic of influenza has passed and opportunities for confirmation since have been scanty. Hall, in 1920, claimed to have produced positive results in rabbits, guinea-pigs and mice by the intravenous, subcutaneous

and intraperitoneal injection of filtered sputum from cases of influenza. The lesions were patchy hæmorrhagic ones. The same author (1926) reported a few results in animals similar to those of Olitsky and Gates, which he obtained by the intratracheal inoculation of unfiltered sputum. None of this work appears to have been pursued in a very critical spirit.

On the other hand, Detweiler and Hodge, although they produced bronchopneumonic lesions in the lungs of rabbits injected intracheally with unfiltered naso-pharyngeal washings, on no occasion obtained any alterations in the lungs of animals injected with filtered washings. They note that special precautions were taken in their experiments to avoid traumatic hæmorrhagic lesions in the lung, which are very readily produced.

It may be remarked that the quantity of inoculum used by Olitsky and Gates was relatively very great—an average of 3 c.c. per rabbit. This would be equivalent to about 120 c.c. for a 12-stone man. The introduction of such a quantity of fluid, of however bland a nature, into the trachea would be calculated to cause a considerable amount of lung trauma and might certainly occasion hæmorrhagic lesions, which in any case are somewhat easily set up in the rabbit's lung. It must be remembered that the lesions depicted by Olitsky and Gates in their experiments with filtered naso-pharyngeal washings were not severe. We may also add that the material injected was a very complex substance, possibly containing bacterial toxins and, as far as we know, any number of filtrable viruses which may exist in the nose and throat of a person sick of influenza, including therewith the herpes virus, whose wide distribution in the mouth and pathogenicity for the rabbit we have already touched upon (p. 146), and whose affinity for lung tissue has been stressed by Levaditi.

With regard to the depression of the mononuclear cell-count, that can hardly, in the conditions of these experiments, be regarded as an exact criterion of the influenzal infection. It is well known that the absorption of foreign proteins, parenterally into the blood, causes marked changes in the leucocyte counts, which vary with many factors, including the type of material and the route of introduction. This phenomenon is the "*crise hémoclastique*"

of French writers, and is seen in its best developed form in acute anaphylaxis. The writer (1922) found that a well-marked reaction of this nature was given by *B. influenzae* toxin prepared according to Parker's method. Unless very closely spaced observations are made upon the leucocytic formula, it would be comparatively easy to be misled by the rather long phase of this reaction in which, with a slightly altered or normal total count, there exists a marked relative and absolute depression of the mononuclear cells.

Turning now to the cultivation and properties of *B. pneumosintes*, we find a good deal of confirmatory work. Loewe and Zeman (1921) obtained growths of this organism in three cases of influenza. Gordon (1922), in this country, stated that he had isolated the organism on fourteen occasions in the course of the examination of twenty cases. He considered the forms seen by him to be identical with those described by Gibson, Bowman and Connor, but his published investigations stop short at the demonstration of the organism. Detweiler and Hodge, in Toronto in 1924, obtained growths of a similar nature in three out of six cases. These were submitted to Olitsky, who described them as being morphologically identical with *B. pneumosintes*. As, however, their attempts at the sub-culture of their organism failed, its position remains open to doubt. Lister, in South Africa, after failure in 1918, reported finding the organism in five out of fifteen cases examined in 1922. This worker also investigated the pathogenicity of his cultures by spraying the nose and throat of nineteen volunteers with growth of the second generation. Only one of these developed what was described to be a typical attack of influenza.

On the other hand, McIntosh (1922), in repeating some of the American work, entirely failed to find any pathogenicity for laboratory animals in the filtered products of cases of influenza, or to get any growths in Smith-Noguchi medium. His results are not open to the criticism that the materials were obtained late in the disease, as in many cases they were taken when the malady was only of a few hours' duration. Branham and Hall (1921) failed to find any growths in Smith-Noguchi cultures from nine

cases of influenza ; the majority of their specimens, however, were collected after the third day of the disease.

In such work negative findings alone do not form a very weighty criticism, on account of the uncertainties which seem inherent in the technique of cultivation as at present practised, and the possibility that a single worker may not at a given time have a batch of medium which is suitable for the purpose. We find, however, that the statement of Olitsky and Gates as to the absence of their organism from the nose and throat in other conditions has been contradicted. Avery has cultivated similar organisms from the normal human throat, and Holman and Crock have done the same, and have also obtained the organisms from rabbits. The last finding, if correct, is a serious difficulty in the way of the unqualified acceptance of Olitsky and Gates' results. These workers have themselves encountered and described a variety of other small filtrable organisms in the course of their work, some easily differentiated from *B. pneumosintes*, others very closely resembling it. There are also notable differences (*e.g.*, in Gram's staining) between organisms described by these workers and those of others in the same field (Gibson, Bowman and Connor). Whatever may be the verdict ultimately passed upon this work, it seems certain that the American authors have directed attention to a group of minute bacteria, possibly widely spread, which had previously escaped observation.

Whilst it is therefore very probable that the *B. pneumosintes* only represents one of a group of small micro-organisms with similar characteristics, the means at our disposal for differentiating between closely similar forms of such organisms are at the present time insufficient, and this may be a source of error, since a number of similar organisms may exist in the same region. The evidence goes to show that a minute organism having the characters of *B. pneumosintes* is a frequent denizen of the nasopharynx in the early stages of influenza. When, however, an attempt is made to extend its significance further the evidence becomes threadbare. Neither upon grounds of pathogenicity, nor of immunity reactions, can its causal rôle in influenza be regarded as proven.

If we attempt to put the claims of Pfeiffer's bacillus side by side with those of the filter-passer, we find that the chief objection urged against the former is the uncertainty surrounding the question of its presence in the uncomplicated disease, and the lack of focal symptoms in this. Here it is that opinion is tempted to incline towards the filter-passer. When we come to the more severe forms of the disease, the evidence takes quite a different turn, for here the Pfeiffer bacillus is admitted on all sides to be the most constant micro-organism, and here the protagonists of the filter-passer themselves state that their organism has disappeared from the scene. These findings would be quite in accord with one of the most acceptable views of influenza, which admits the importance and pathogenicity of Pfeiffer's bacillus, but limits its activities to the complicated forms of the disease; the prime agent in opening the respiratory passages to its action being a filter-passer with a propensity for damaging the lung.

As regards pathogenicity, there are no difficulties about Pfeiffer's bacillus. It is definitely pathogenic for laboratory animals and, as Parker has shown, produces a very poisonous toxin in young cultures. The opinion is expressed by McIntosh, and we entirely agree with this view, that this toxin may be responsible for many of the clinical phenomena of influenza. That the bacillus is capable of producing lesions in the respiratory tract of animals which are very similar to those of influenza has been abundantly proved by Blake and Cecil. The organism occurs in the essentially similar lesions in man and, moreover, provokes the presence of antibodies in his serum in a large proportion of cases of influenza. The real question at issue is whether it initiates these lesions or merely participates in them. The *B. pneumosintes*, on the other hand, and for the purposes of argument we here assume that this organism is the agent which is responsible for the pathogenic effects of filtered influenzal sputa, is extremely disappointing as a pathogenic agent; it cannot hold a candle to Pfeiffer's bacillus in this respect, and in attempts to produce the experimental disease in man it seems that it must also yield place to that organism.

It is difficult to find a problem more complex than that of the ætiology of influenza. There is no firm ground anywhere, even

such matters as the characteristics of Pfeiffer's bacillus, or of the disease itself, being uncertain. It is a very hard task to investigate a disease which may entirely change its characters within six months. For this reason, if for this alone, it must be the first essential in any investigation of the disease that this should be carried out upon epidemic influenza. Uncertain though we are of the position of the proposed claimants to its ætiology, the problem of influenza is much better defined than it was prior to the production of the mass of confused work we have been reviewing. The issues have been made clear, and we must wait for the next appearance of the undoubted disease in epidemic form for the problem to be solved

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## THE COMMON COLD

If there is some uncertainty whether influenza is a specific infection, or whether infections with a number of different organisms can produce the influenzal syndrome, the question of the common cold is in even worse plight. Colds vary a great deal in their symptomatology, not only from person to person, but from cold to cold in the same individual. "This isn't one of my usual colds," is an expression we have all heard, and it would be a bold assumption to conclude that every case of "cold" is due to a single and same organism. Nevertheless this is often done.

The modern view of the common cold is that it is due to a filtrable virus, a suggestion which dates from Kruse's experiment in 1914, in which he instilled into the nostrils of twelve individuals a Berkefeld filtrate of the diluted nasal mucus of a subject suffering from acute coryza. Four of the volunteers contracted colds. In a second experiment he obtained a similar result.

Foster (1916-17) confirmed Kruse's results, and from Berkefeld filtrates of the infective nasal discharges claimed to have grown in Smith-Noguchi medium organisms resembling the "globoid bodies" of Flexner and Noguchi (p. 184). More recently Olitsky and McCartney (1923) obtained the same results as Kruse in infecting volunteers with filtered nasopharyngeal secretions taken during the first eighteen hours of the attack. They found that at a later period the secretions were no longer infective. In cultures by the methods which Olitsky and Gates had developed for the isolation of *B. pneumosintes*, they obtained growths of a variety of organisms, upon the significance of which they were unable to pronounce. They were, however, unable to confirm Foster's results, which are generally regarded as being due to that observer having been misled into interpreting protein precipitates, which are inseparable from the Smith-Noguchi method, and have over and over again led into error, as micro-organisms. It may be

recalled that in their earlier experiments in the isolation of *B. pneumosintes* in influenza, Olitsky and Gates used a number of cases of coryza as controls. At that time they failed to get any growths confirmatory of Foster's findings. Schmidt, who made 196 filtrate inoculations, obtained no results which would support the theory of Kruse, and Branham and Hall (1922) failed to obtain any growth in cultures from this condition. A yet later experiment is that of Robertson and Groves (1924), who sprayed the noses of 100 volunteers, using in each case about 1.0 c c. of a Berkeley filtrate of diluted and homogenised nasal washings from cases of acute coryza. One volunteer developed coryza, one influenza, two laryngitis, and ninety-five were none the worse during an observation extending over twelve days. The few illnesses which occurred during the period of the experiment were attributed to chance infections. It will be seen from these observations that the balance of the experimental evidence is rather against the filtrable-virus view of this condition.

One of the marked peculiarities about the common cold, if due to a single micro-organism, is the absence or extreme shortness of the subsequent immunity. There is, however, at least one parallel for this amongst the filtrable viruses, in the case of foot-and-mouth disease. If a filtrable virus is to blame, and if it is only active in the early stages of the complaint as the experiments of Olitsky and McCartney seem to suggest, then the long-drawn chronic phase of the cold is presumably due to secondary infection with nose organisms, the virus here acting in the same way as the influenza virus is conceived of acting. This matter is of some importance in connection with the use of "catarrhal vaccines" in the prophylaxis of the cold. Although these are in many cases enthusiastically advocated, the few well-controlled experiments which have been carried out do not go to show that they are of any value in decreasing the incidence of colds in a community. The author, with members of the Manchester University staff, carried out an experiment upon the students at that school in the winter of 1924-25, dividing the volunteers, of whom there were 286, into two groups similar in every respect, and inoculating one half of them with a preventive vaccine, the other group acting as

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controls. The vaccine in use was one of the grape-shot order, issued by a highly reputable commercial firm and used in this instance with the cognisance of their scientific director. It contained most of the known nasopharyngeal organisms, including *B. pfeifferi*. Three doses of vaccine were given to each of the inoculated persons. The experiment lasted from November, 1924, till the end of July, 1925. Carefully kept records showed the following results —

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		Number of Per- sons in each Group	Total Number of Colds Recorded	Number of Colds per Person in the Period of Obser- vation
Inoculated	.	188	254	1.84
Uninoculated	.	148	202	1.36

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The result indicated that no effect upon the incidence of colds was produced by the inoculation. Similar observations have been published by von Sholly and Park, and by Jordan and Sharp, in America; both of these, especially the former, worked with much larger groups of persons. The only loophole of escape from the logic of these figures is that it is conceivable, as we have already suggested, that colds are syndromes having different bacteriological bases. In such a case a set type of vaccine may be quite unsuitable, and the possibility remains, remote though it may be, that an autogenous vaccine made during the currency of a previous cold may benefit an individual person.

The failure of these different groups of workers to lower the incidence of colds by the use of vaccines, in so far as it has any bearing upon the problem at all, goes to suggest that the causal agent has been uniformly missing from the vaccines, and for that reason lends some support to the filtrable organism hypothesis. If the cold were due to such a virus, then no amount of vaccination with other organisms would affect its incidence, although an effect upon severity might result if this were dependent upon secondarily

invading organisms of the types present in the vaccine. We have no evidence of such a result, but it is a question much dependent upon individual opinion, and therefore one upon which it is extremely difficult to obtain reliable information.

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## CHAPTER X

### DISEASES ASSOCIATED WITH RICKETTSIA BODIES

IN 1910 Ricketts and Wilder described the presence of minute bodies, resembling bacteria, in the intestinal canal of lice which had fed upon typhus fever patients. Similar bodies have been found in the wood tick (*Dermacentor venustus*), which is associated with rocky mountain spotted fever, and in the body louse in cases of trench fever. The more important experimental facts in connection with these diseases are resumed in the pages which follow.

## TYPHUS FEVER

The experimental investigation of this disease was made possible by Ch. Nicolle, who, in 1909, having previously failed to infect monkeys with the blood of typhus patients, succeeded in infecting a chimpanzee by the subcutaneous injection of blood. By passage from this animal he was then able to infect bonnet monkeys and to transmit the disease serially from monkey to monkey. In the same series of experiments he demonstrated the protective action of human convalescent serum for the monkey and also established the very important point that the disease was transmissible from animal to animal through the agency of human body lice. Nicolle found that the blood was virulent during the whole of the pyrexial period and for the first day or two of convalescence as well. The preventive and curative antibodies made their appearance in the serum on the tenth or twelfth day after the temperature had subsided; they were found to be somewhat fleeting, and had generally disappeared fifteen to twenty days later. These animal experiments were soon confirmed both by Anderson and Goldberger, and by Ricketts and Wilder, in the United States, who found that positive results could be obtained with the ordinary *Macacus rhesus* monkey if 1.0 c.c. or more of virulent blood were injected; smaller doses may give negative results, which probably accounts for those of Nicolle. The blood should be taken before the end of the tenth day of the disease. Wilder (1911) was able to transmit typhus to the monkey from human sources by means of lice, and to show, a fact also noted by Nicolle, that infection could occur in these animals, as judged by the subsequent appearance of immunity, without any clinical evidence of the disease being manifest. His experiments also showed the infectivity of the intestinal contents of the louse, and that in this material the virus was more active than in infected blood. The experiment of the transmission of typhus to man by

louse bites, and by the inoculation of the crushed bodies and nits of lice fed upon a typhus patient, was accidentally made by Sergeant, Foley and Vialatte, who were working upon relapsing fever and were ignorant of the presence of a typhus infection at the outset of their experiment. The transmission succeeded perfectly in all three instances in which the attempt was made!

Lice, according to experiments by both Wilder and Nicolle, do not as a rule become virulent until a certain latent period, of about seven days, has elapsed after the infecting meal. The excreta of the animal conveys the infection and nits of the first generation may be contaminated, but there is little positive evidence that the virus is conveyed to the second generation of lice, in contradistinction to what pertains in recurrent fever

It is generally held that the infection in typhus is conveyed by the bite of the louse. Arkwright and Bacot (1923), who were experimenting upon the infectivity of louse excreta, which they found remained active for eleven days at room temperature, both became infected with the disease, although neither of them had been in contact with typhus cases or been bitten by lice. They considered that the infection is only doubtfully directly implanted by the bite of the louse, and that it probably enters the body more often through minor cuts and abrasions. It may also be self-inoculated by scratching, as is considered by English workers to be the usual mode of infection in trench fever, and the possibility of conjunctival contamination cannot be ignored.

A certain amount of further information has been established about the virus of typhus. It is present in the plasma as well as in the whole blood, but only occasionally has the serum, obtained by clotting, been found to be infective. The virus is comparatively easily killed, being destroyed by fifteen minutes' exposure to a temperature of 55° C. and by drying for a short period.

The evidence for the filtrability of the virus of typhus is distinctly weak. It has been held to be filtrable on the grounds that inoculation of filtered serum might render an animal refractory to the virus. The experiment is, however, open to other interpretations, and there does not appear to be any convincing account of

the transmission of the disease in series with filtered material, derived from either the diseased subject or from the infected louse.

Nicolle further advanced the means of investigating typhus by showing the possibility of infecting guinea-pigs. These animals are not all susceptible to the infection, which shows itself by fever only, occurring as a rule through the sixth, seventh or eighth days after inoculation, when the temperature rises two or three degrees above the normal. Unless the temperature is regularly observed the presence of the infection may easily be missed. Its specific nature is demonstrated by the fact that the blood of such guinea pigs will re-infect monkeys, after a series of passages through the first-named animal. The typhus virus is present in all the organs of the infected guinea-pig, but seems especially concentrated in the brain and suprarenal capsules.

Though not an animal of choice for the investigation of the disease, by virtue of the relative mildness of the infection and a degree of uncertainty about the results, the guinea-pig offers obvious advantages, especially in the matter of preservation of the virus, and it has been widely used in experimental work. It is not suitable for experiments with lice, since its blood appears toxic for these creatures, which succumb after a meal of it. Nicolle has shown that a certain number of inoculated guinea-pigs give no febrile reaction, but, nevertheless, harbour the virus in their blood and tissues during the period in which positively reacting animals would be found to be febrile. On this account seemingly negative results do not mean a loss of the virus, since animals subsequently injected with the blood from those failing to show a reaction usually react positively. To this condition of afebrile infection Nicolle and Lebaully have given the name of "typhus inapparent," which may be translated as "undeclared typhus."

The phenomenon has greatly interested these workers, not only for the special case of typhus, but on account of its bearing upon epidemiology generally, and the question of "formes frustes." They have shown that the typhus virus comports itself regularly in this way when introduced into the rat, and have succeeded, on two occasions, in transmitting the disease in the undeclared form

through as many as twelve passages in rats. Results of a like nature were obtained with mice and the gerbille.

Nicolle finds that animals such as the guinea-pig acquire only a short-lived immunity as the result of an undeclared infection, an immunity less lasting than that obtained where an obvious febrile reaction results. He also finds that the infection may assume its undeclared form in partially immune animals. He thinks the matter significant from the epidemiological point of view, since the typhus of infancy and childhood is, as is well recognised, a benign malady, and therefore is probably succeeded by an immunity which is only partial. In localities in which outbreaks of the disease are frequent, or in which it is endemic, the suggestion is made that in many cases the infection may exist in the undeclared form, or masquerade under the guise of a trivial ailment, in partially immunised persons, a possibility which, if realised, would contribute to an important extent to the spread of the disease.

**Nature of the Infecting Agent.**—Innumerable claimants have appeared for the ætiological rôle in typhus. The proteus organisms which Wilson and Weil and Felix have found to be agglutinated so specifically in this disease as to yield a diagnostic procedure of real value, are generally admitted to be of no ætiological importance. Two agents only require any consideration at the present moment—the bacillus of Plotz, and the Rickettsia bodies. The Plotz bacillus was described by that author in 1914 as a short gram-positive organism, which he obtained from the blood of typhus patients by a special anaerobic technique, which consisted in taking the blood directly into tall tubes of ascitic-glucose-agar. Positive blood cultures were obtained in a high proportion of cases, and Plotz, Olitsky and Baehr further described agglutinating and complement-fixing bodies for the organism in the serum of convalescents: a matter to which we do not now attach great weight on account of the well-recognised tendency for typhus blood to contain non-specific antibodies.

The case made out for this organism was at the time seemingly so good that immediate opinion was inclined to regard it as the genuine causal agent of typhus. Workers in the Serbian epidemics did not, however, succeed in obtaining the organisms, and when

bacilli were grown from typhus cases under the conditions laid down by Plotz they were not always found to be of the same nature. Finally, the increasing scepticism was brought to a head by the criticisms which Olitsky (1921) directed against this organism. He states that antibodies are found in the blood of typhus patients not only against the *Proteus* group (Wilson, Weil and Felix), but sometimes against *B. pyocyaneus*, *M. melitensis*, or the typhoid bacillus, any of which may be more definite than those found for Plotz's bacillus. He also believes that the guinea-pig



FIG. 18.—*R. prowazeki* within the swollen and degenerate endothelial cells of a skin arteriole, in typhus (After Wolbach, Todd and Palfrey)

infections, which were claimed to have been occasioned by this organism, were non-specific. Since Olitsky had originally been associated with Plotz in sponsoring his bacillus, such a reversal of opinion may be taken to dispose of its claims effectually.

**Rickettsia in Typhus.**—After their discovery by Ricketts and Wilder in 1910, the presence of rickettsia bodies in the blood in typhus cases was announced by Hegler and von Prowazek in 1918, and their existence in infective lice was definitely confirmed by Sergeant, Foley and Vialatte in Algiers in 1914. In human tissues various workers described the bodies, always scanty, in the blood, the leucocytes, and the Kupffer cells of the

liver. Stevenson and Balfour (1921) found indistinguishable granules in small numbers in formalin-fixed tissues, but were somewhat sceptical as to their exact nature and significance. An elaborate study was made by the Typhus Research Commission of the League of Red Cross Societies in Poland, under the direction of Wolbach, Todd and Palfrey (1922). They found rickettsia to be present in the vascular endothelial cells (Fig. 13), but never in the muscle fibres of the vessels, as is the case with the corresponding bodies in Rocky Mountain Spotted Fever. It was rare to find cells crowded with rickettsia, but common to find a few. They were also seen within the cells of the perivascular nodules. The commission established their presence in the skin in every case in which the post-mortem examination was made before the eleventh day of the disease and in which the body was in a fresh condition. The same formations were also on occasions discovered in the kidneys, testes and brain. They failed to find the bodies in a large variety of control tissues, and state with assurance that

the rickettsia seen in this condition are pathognomonic of typhus, and that they are indistinguishable from the rickettsia seen in the infected louse. In the louse the bodies are found almost exclusively in the epithelial cells lining the gut, within which they appear to undergo proliferation (Fig. 14).

Bacot and Ségal (1922), and Arkwright and Bacot (1928), found that the infectivity of suspensions of blood platelets from typhus patients for guinea-pigs was very high, and they also found that the introduction of this material, per anum, into normal lice resulted in their becoming infected with *R. prowazeki*. The infected lice at the same time acquired the power of causing typhus

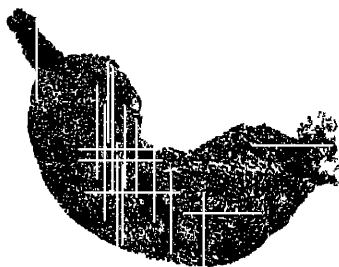


FIG. 14 — *R. prowazeki* within a swollen epithelial cell of a louse's mid-gut. One end of the cell is filled with a granular mass of rickettsia, at the other they are thread-like. (After Wolbach, Todd and Palfrey)

in the guinea-pig. In examining lice fed on twelve healthy persons, for control purposes, they failed to find rickettsia, but lice fed on two others showed a rickettsial infection over a short period. These same persons subsequently failed to infect other lice. The rickettsia associated with healthy persons differed in certain respects both from *R. prowazeki* (typhus) and from *R. quintana* (trench fever), and somewhat resembled a form described in Poland by Weigl under the name of *R. rocha-lima*. This type is intracellular and resembles *R. prowazeki*, but is non-pathogenic and shows no immunological relationship to the latter. On account of their inability to obtain subsequent rickettsial infection from these persons, Arkwright and Bacot concluded that the lice had derived their organisms from some other source. Their experiments upon the virulence of this intruding strain of rickettsia were generally negative, but were not able to be carried to a conclusion as both workers contracted typhus, of which Bacot died.

**The Weil-Felix Reaction in Typhus.**—This laboratory aid to clinical diagnosis is now so well known as scarcely to need notice. It was discovered independently by Wilson, of Belfast, in 1910, and by Weil and Felix, in 1916, that certain coliform organisms, isolated from typhus cases, were agglutinated by the serum of typhus patients and by these only. The best known of these organisms is the *Proteus* X. 19, of Weil and Felix, and the reaction, although not quite fairly to other and earlier observers, is generally known by their name. At first the tendency was to regard the organism as having an aetiological relationship to the disease, but that view is no longer held, and the reaction is looked upon as one example of the rather rare and curious phenomenon of heterologous antibody action.

The diagnostic value of the reaction has been confirmed on all sides. It begins to appear early in the disease and is positive by about the fifth day in 50 per cent. of cases of typhus, and very shortly becomes positive in over 90 per cent. of cases, in a serum dilution of 1 in 25 and upwards. Sometimes the titre rises to 1 in 10,000 or even more, and in a case mentioned by Ledingham reached 1 in 80,000.

The tendency for typhus sera to cause heterologous agglutina-

tion has now become well recognised, and this effect is not restricted to the *proteus* group, although the organism of Weil and Felix is the one generally adopted for the clinical test. There is little doubt that it is the presence of this non-specific quality which has so repeatedly given grounds for the belief that one or other bacillus might be the causal agent of the disease, beliefs which have been of short duration, and which the modern discovery of the possibilities of experimental investigation of the disease have swept away. None of the various bacterial claimants is able to cause the specific febrile reaction in the guinea-pig, and none of them protects that animal against a subsequent dose of virulent blood. In one respect, however, the Weil-Felix reaction stimulates the further curiosity of the bacteriologist, and that is its dependence upon the O antigenic element. It therefore appears to be excluded from the category of group agglutination effects, which it is generally admitted are independent of this antigen. The stimulation of specific agglutinins for X. 19 is therefore due to either some incidental pathogenic effect, which is produced by this organism in almost every case of the disease, or to factors in antibody production at present unrecognised.

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## TRENCH FEVER

A disease of the war. In the summer and autumn of 1915 the number of cases of rather indeterminate pyrexia occurring amongst the troops on the Western front became enormous, and those who had the opportunity of observing these over a sufficiently long period soon recognised that they were dealing with a hitherto undescribed entity, chiefly because of the typical recurrent type of pyrexia presented by many of the cases. This was the most striking clinical feature, and the one which from the first suggested a new disease rather than a modified form of typhoid, which it was at one time suspected of being. Once attention had been focussed on the malady from this angle the breadth of the clinical picture was found to be considerably greater, and to include also cases which had pyrexia of a continuous form. The only other distinctive clinical features were the rather characteristic and sometimes severe shin pain and a tendency to relapses.

The disease soon appeared upon the German eastern front (Wolhynian fever) and the Austrian fronts, and was also noted amongst Italian troops in 1917. The symptomatology of the disease became aggravated as time wore on, and those who investigated it in England, and especially the experimentally transmitted disease, studied a much more severe malady than that seen in the forward areas in 1915. Enlargement of the spleen, tachycardia, arrhythmia, anaemia, and chronic ill-health, were often notable features of such cases. The disease never gained any hold upon civilian populations, and at the present time would appear to be extinct—although the diagnosis of sporadic cases would be a matter of uncertainty and difficulty.

The laboratory investigations at the outset were in all cases of a negative nature. Typhoidal infections were readily excluded ;

the blood was devoid of organisms when cultivated by ordinary methods; and blood counts showed nothing typical save for punctate basophilic stippling of the red cells, which was found to be present in a considerable number of cases.

In 1916 McNee, Renshaw and Brunt reported the first attempt to elucidate the nature of the infection. They succeeded in conveying the disease to man by the injection of whole citrated blood and of washed red corpuscles; but not by plasma, serum, or lysed and filtered blood. These results were confirmed by the American Red Cross Commission in 1918, working with facilities which were lacking to the earlier investigators. It was found, however, that the virus might be present in the plasma, although it could be removed from it by centrifugalisation.

The view soon came into being, both in this country and in Germany, that the disease was a louse-borne one, and isolated experiments by a few workers supported this view. The matter, however, was not definitely settled until the investigations of the commissions appointed by the British Army authorities and by the American Red Cross, both of which incriminated these creatures as the carriers of the infection. Both concluded, as a result of exhaustive experiments, that the body louse, *Pediculus humanus*, was the agent concerned in the active transmission of the disease. The British workers further concluded that *Pediculus capitis* was also culpable, and that the infection was essentially present in the excreta of the louse. They believed that scratching caused the lesion which permitted the virus to enter, and were somewhat dubious if the louse bite was a route for this. The American commission reached a rather different conclusion on this matter, reversing the importance of these alternative modes of infection and placing the bite first, with scratching and rubbing in of infected matter as a bad second.

Both sets of investigators agreed as to the non-transmission of the infection to the second generation of lice. As had been found by Nicolle for typhus, a latent period of some days was necessary before the infected louse became able to transmit the disease. Once infected, the lice retain the infection whilst they remain in existence.

The presence of rickettsia bodies in trench fever infected lice was discerned by Topfer, da Rocha-Lima and Munk, and others in Germany in 1916-17, and by Arkwright, Bacot and Duncan in this country (1918). Rickettsia were found to develop in clean lice which were fed upon trench fever patients and maintained at or about body temperature (Fig. 15). Lice fed on healthy uninfected individuals did not develop these bodies. The infectivity of the



FIG. 15 —*R. quintana*. Film of excreta of an infected louse, fifteen days after feeding upon a trench fever patient —(Arkwright, Bacot and Duncan.)

patients for lice was very prolonged, extending to well beyond convalescence; it was found that where lice fed under such conditions came to contain rickettsia, they at the same time became infective for man.

The rickettsia bodies have not been demonstrated in the human subject in this disease, as on account of its non-fatal nature the opportunities of searching for them in the tissues are practically nil, whilst the search for a few minute particles of this nature in the blood is too full of fallacies to be reliable.

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## ROCKY MOUNTAIN SPOTTED FEVER

Another disease in which rickettsia have been implicated is Rocky Mountain Spotted Fever, which has many analogies with typhus. The disease is, however, readily communicable to guinea pigs by inoculation with infected blood ; it produces a severe illness in them which is often fatal. King and Ricketts (1906) finally proved, what had long been suspected, that the disease is conveyed by a wood tick, *Dermacentor venustus*, and that it could be transmitted from man to animals by the bite of this tick. The infection appears to be transmitted to the egg. In this disease, as in typhus and trench fever, the contagion is not filtrable.

Ricketts, in 1909, described the bodies now known by his name, in human blood, in the blood of infected guinea-pigs and in the tissues and eggs of ticks, although his account was confused by failure at that time to differentiate these bodies from bacteria which were also present. Wolbach (1916-19) confirmed these findings in detail, and with a more specific conception of the rickettsia bodies, which he was able to demonstrate in the infected ticks. The infection is a very widespread one in the tick, the gut, salivary glands, reproductive organs, brain and muscles, all showing the parasite. There is a complete absence of cellular reaction (*cf.* Cowdry, p 227) Wolbach also noted the multiplication of the rickettsia in the body of the ticks and their presence in the eggs. He studied the histological lesions of the disease with especial care and found the rickettsia to be localised almost exclusively in the endothelial cells and in the muscle cells of the tunica media of the damaged blood vessels of the corium and external genitals, both in man and in the guinea-pig. Wolbach concluded that the parasite, rickettsia, was pathognomonic of, and inseparable from, ticks capable of transmitting this disease

The point having been raised that the bodies described were really mitochondria, Nicholson (1928) went over the same ground

as Wolbach and confirmed in all details his finding of the parasite in the tissues of infected guinea-pigs. At the same time he carefully differentiated them from mitochondria, phagocytosed blood-pigment, nuclear debris, and other known cellular constituents. Nicholson found that the appearance of the rickettsia in the early stages of the guinea-pig infection was generally in a diplobacillary form, whilst the single bacillary type predominated towards its termination.

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## HEARTWATER

This cattle disease of the Transvaal (veldt sickness) gains its name from the characteristic pericardial effusion which occurs in the sick animals. The disease is transmissible to sheep and goats, as well as to cattle, by inoculation of the blood of a diseased animal, and has a mortality of about 50 per cent.

Theiler showed that the disease was indubitably due to a *contagium vivum* in the blood, and that this was not of a filtrable

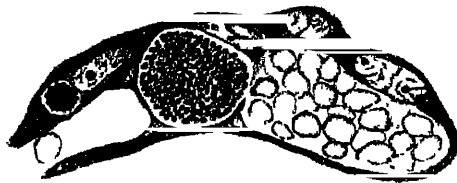


FIG 16—A vessel of the cerebral cortex in heartwater disease, showing a clump of rickettsia within a swollen endothelial cell. (After Cowdry.) (The artist has slightly exaggerated the proportions of the rickettsia.)

nature. Cowdry (1925-26), at the suggestion of Theiler, investigated the disease from the point of view of a possible rickettsial parasite, and succeeded in finding these bodies in large numbers in the capillary endothelium of the diseased animals' tissues; more particularly in the renal glomeruli and in the superficial vessels of the grey matter of the cerebral cortex (Fig. 16). The rickettsia are only to be found in fresh tissues, disappearing in a few hours after death. The same observer found that these bodies underwent multiplication in the endothelial cells, which appeared to tolerate their presence well. Eventually, however, the cells might be burst by the pressure of the parasites, and the latter were then discharged into the blood stream.

It had previously been known that the disease was transmitted from animal to animal by a tick (*Amblyomma hebraeum*) and that the infection did not pass to the egg. Cowdry succeeded in demonstrating a rickettsia, identical in appearance to that found in the tissues, in this tick, more especially in the intestinal epithelial cells. He did not find it in other ticks examined as controls. The parasite has been named *Rickettsia ruminantium* by Cowdry who, on account of its invariable presence in the disease and in the infective arthropod vectors, considers it to be the causal agent of heartwater.

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#### Heartwater

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## THE GENERAL FEATURES OF THE RICKETTSIA BODIES

The rickettsia bodies were first found by Ricketts in Rocky Mountain fever, in 1909, and later were identified by Ricketts and Wilder in typhus cases, in 1910. They have since, as we have seen, been described in trench fever and in heartwater, all louse- or tick-transmitted diseases.

In none of the diseases with which we are now concerned is the virus filtrable. This is fairly well established. Allowing for the known pitfalls in a negative filtration experiment, we may therefore assume that the organism should be just within the range of microscopic visibility. It is present in the disease ; it is present in the louse ; it should be visible . has it been seen ? The only formations yet described, which are visible in both hosts, are the rickettsia bodies. The dimensions of the pathogenic forms are about  $0.3-0.5\mu \times 0.3\mu$ , or a little larger, and their presence in large numbers is constant in the infective arthropod. Their prevalence in the human host, or in the diseased animal, is less obvious ; but with increasingly careful research and better knowledge of the technique necessary for their adequate fixation and staining, their demonstration is becoming more common. They are, moreover, characteristic of the infected arthropod as distinct from the normal. In the latter they do not occur in any significant frequency, and in some cases are not found at all. We may, to some extent, discount many of the earlier criticisms of the rickettsia, based upon their supposed presence in normal lice, as at the time these were made they had not been sufficiently differentiated from non-specific granules and, further, many of the observations upon which criticisms have been based were made at a time when trench fever was rife and had not then been recognised as a rickettsial disease. This is now known, and it is further recognised that the blood may continue to cause the development

of rickettsia in lice for long periods after the disease has been recovered from.

The study of the rickettsia owes much to the entomologist, da Rocha-Lima, who, in 1916, accepted this form as a living organismal parasite, which he believed to be a strongyloplasma, as the causal agent of typhus fever. He coined for the species the name *Rickettsia prowazeki* in homage to the two workers, out of the many who have lost their lives in the study of typhus, who had contributed most to the recognition of this infective agent. Since that time the number of species of rickettsia has grown prodigiously, and it is now realised that they are exceedingly common inhabitants of the arthropod group. This raises no *a priori* difficulty in their acceptance as pathogenic agents, but in some ways helps it, since it is almost a law in bacteriology that saprophytic micro-organisms are commonly encountered in the same sites as pathogenic ones, and a study of the former often aids in an understanding of the more exacting pathogenic forms.

The criterion of the rickettsia bodies, given by Cowdry (1923), is terse: "Gram-negative intracellular bacteria-like organisms found in arthropods." It indicates the looseness and elasticity of the group within which these bodies lie. In general, rickettsia may be said to be small organisms which stain ill with the ordinary bacterial dyes, but fairly well with Giemsa. They are less sharply defined and outlined than are bacteria. Their shape is very variable, being sometimes in the form of tiny filaments, sometimes bacteria-like, sometimes coccal; but it is characteristic that at one time or another all varieties and strains seem to assume the minute coccal or diplococcal form. The recognition of isolated units is difficult, of masses easy to the experienced observer. They are generally described as non-motile, but Arkwright found that the filamentous forms of *R. lectularia* were motile. The parasites are found in a great variety of arthropod hosts, including lice, ticks, bugs, mosquitoes, sand flies, etc., but only four types are at present recognised as pathogenic, these being, in order of their discovery: *Dermacentorzenus rickettsia* of Rocky Mountain spotted fever; *Rickettsia prowazeki* of typhus, *R. quintana* of

trench fever, and *R. ruminantium*, recently described by Cowdry in heartwater disease of cattle. These minute bodies develop in the gut of the arthropod host, where they may be seen both within the epithelial cells and free in the lumen, as in typhus; or sometimes almost exclusively extracellular, bordering the lining epithelium of the gut, as is the case with *R. quintana*. The parasites are discharged in the insects' dejecta, and are there seen in enormous numbers. After a feed of infective blood a certain period elapses before they become discernible in the louse. In trench fever Arkwright did not find them before the fifth day, although they were well in evidence in eight or ten days and attained a maximum by about the end of the third week. The lice require to be kept in the warmth and regularly fed for the proper development of the rickettsia to take place. In certain cases, to which spotted fever conforms and typhus is a notable exception, the rickettsial infection is transmitted through the eggs to the next generation.

The exact nature of the rickettsia bodies has been, and still is, very largely a matter for conjecture. Though resembling bacteria in many respects, they have not been cultivated upon artificial media, with the exception of a non-parasitic form, *R. melophagi*, met with in sheep keds, which Noller claimed to have cultivated, this was substantiated by Hertig and Wolbach (1924). The organism grew with difficulty upon agar media, and in culture was indistinguishable from a bacterium. The case has been urged in different quarters that the rickettsia are mitochondria, cell granules; the granular products of digested blood (Woodcock), non-specific granules, and the like. In his masterly review of the existing types of rickettsia, and their distribution, Cowdry (1928) very thoroughly examined the position and pointed out the following important considerations bearing upon their nature:—

- (1) That though most frequently met with in blood-sucking insects, rickettsia are not restricted to these, and are also to be found in insectivorous and vegetarian forms.
- (2) That the presence of rickettsia cannot be linked with any particular physiological condition in the habits of the insect host.

- (3) The parasites are met with in cells which are non-phagocytic, or are only very slightly so
- (4) A distinction between rickettsia and mitochondria is readily made by appearance, distribution, and staining reaction.
- (5) That there are no grounds for identifying rickettsia with chlamydozoa.

The marked variations in size, shape, and appearance of different rickettsia has suggested that certain of the forms seen are merely rather special types of bacteria. This view needs to be looked at in the light of some observations made by Arkwright and his associates upon *R. lectularia* of the common bed bug. The appearances presented by this organism are remarkably pleomorphic and lend support to the opinion that they represent recurring phases which constitute a life cycle. Arkwright also observed a granule emission, in the case of the thread forms of *R. lectularia*, and believes that the granules may represent the coccal forms of the parasite seen at other times. Wolbach (1919) also noted a somewhat similar series of morphological changes undergone by the Rocky Mountain spotted fever rickettsia which, by their occurrence in sequence, also suggest a life cycle or at least development phases. He considered that the parasite might be present in the leucocytes of the patient's blood in this disease. Nicolle, who has not been by any means an advocate of the rickettsial theory, similarly believes that the virus of typhus exists in this situation. One final set of observations may be noted, which has a bearing upon the specific individuality of the rickettsia, if not upon their ætiological significance. Ricketts, in the early days of his investigations, noted agglutinins for these bodies in the serum of patients sick of Rocky Mountain spotted fever and in the blood of immunised guinea-pigs. Ledingham, working with the trench fever rickettsia, found that rabbits inoculated with the emulsified excreta of infected lice developed specific agglutinins for *R. quintana*, whereas rabbits inoculated with the excreta of normal lice did not develop any such agglutinins. These facts are evidence of some weight in favour of the specific individuality of the rickettsial bodies, a conclusion which we have already reached upon other grounds. If immunity

Comparison of *Rickettsia* in Arthropod Vectors (After Cowdry)

	Spotted Fever ( <i>Dermacentorzenus rickettsia</i> )	Typhus Fever ( <i>R. prowazeki</i> )	Trench Fever ( <i>R. quinana</i> )	Heartwater ( <i>R. ruminantium</i> )
Morphology	Pleomorphic (a) Extracellular bacillus-like, 0.6-1.0 $\mu$ in length (b) Intracellular minute rods with chromatoid granules (c) Larger, lanceolate, paired forms. "Less bacterium-like than any of the rickettsiae." Does not occur in thread-like or filamentous forms as does <i>R. prowazeki</i> . Intracellular and intranuclear multiplication	Pleomorphic 0.3 $\times$ 0.4 $\mu$ for single elements, and 0.8 $\times$ 0.9 $\mu$ for double	Pleomorphic 0.8 $\times$ 0.3 or 0.8 $\times$ 0.5 $\mu$ , round, oval, diplococcal, or bacillary with stained poles. More uniform in morphology, plumper and more definitely oval than <i>R. prowazeki</i>	Pleomorphic Cocci 0.2-0.3 $\mu$ in diameter, bacilli 0.2-0.3 $\times$ 0.4-0.6 $\mu$ , and diplococci 0.2 $\times$ 0.8 $\mu$ . No filamentous forms observed.
Phases of multiplication		Multiples exclusively within the cells of the louse	Multiples within the lumen of the intestine of the louse and on the surface of the epithelial cells	Multiplication takes place within the intestinal epithelial cells and peritropics in the intestinal lumen
Microchemical reactions	Gram-negative Form (a) stain pale blue with Giemsa Form (b) stain blue with deeply coloured chromatoid granules Form (c) are purple Outlines less sharp than those of most bacteria, not acid-fast Very simple in well-fixed tissues and smears, and much easier than in infected animals	Gram-negative Stains feebly with basic aniline dyes. Pale blue with Giemsa	Gram-negative Easier to stain than <i>R. prowazeki</i> , stains purple with Giemsa	Gram-negative Easily stained dark blue by Giemsa
Detection		Ditto	Ditto	Ditto
Motility		Ditto	Ditto	Ditto
Position	Absent Extracellular, intracellular and intranuclear	Absent Extracellular and intracellular. Never intranuclear	Absent Chiefly intracellular. Never intranuclear	Absent Chiefly intracellular. Sometimes extracellular. Never intranuclear

reactions can be regularly obtained between the blood of infected animals and emulsions of the rickettsia, the antigenic rôle of the latter, and their causal rôle in the clinical conditions, will be immensely strengthened. Epstein (1922) claims that this is the case in typhus.

An excellent tabular comparison of the pathogenic types of rickettsia is given by Cowdry and partially abstracted here. For a fuller description, and a similar comparison based upon their appearances in mammalian tissues, the reader is referred to the original papers (*Journal of Experimental Medicine*, 1925, Vol. 42, pp. 268 *et seq.*).

For work upon the rickettsia freshness of tissues and good fixation are essential Zenker's fluid is universally recommended as a suitable fixative, as is also Regaud's. The composition of the latter is:—

8 per cent. potassium bichromate	.	.	.	4 parts
Commercial formalin	.	.	.	1 part

Giemsa's stain is that which has been the most widely used for their demonstration.

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## CHAPTER XI

### MEASLES

THE experimental investigation of measles may be said to have begun with the experiments of Home, in 1759, who applied dressings soaked in blood which had been abstracted from patients suffering from this disease to wounds made in the arms of other healthy persons, and who believed that he had produced measles thereby. The disease, if measles it was, was very much modified in type, and later workers have generally disputed the identity.

The more modern phase of the artificial transmission of the disease dates from Hektoen's experiments in 1905. This worker successfully conveyed the disease to two medical students, by the subcutaneous injection of the blood of patients collected upon the fourth day of the illness. Whilst cultural experiments failed, Hektoen was able to demonstrate that the virus remained alive for at least twenty-four hours in ascitic broth. Anderson and Goldberger (1911) carried the matter somewhat further by transmitting the disease to monkeys, and they also claimed that by the subcutaneous injection of nasopharyngeal secretions, collected in the early stages of the disease, they could infect these animals, an observation which confirmed the earlier ones of Meyer upon the infectivity of the nasal secretions in man. They also, at the same time, succeeded in transmitting the virus from monkey to monkey. Although Nicolle and Conseil (1911-20) also claimed to have successfully infected monkeys, both Jurgelunas, in Germany, and Sellards and Wentworth, in America, entirely failed to reproduce either these results or those of Hektoen. In reviewing the accumulated positive findings at this period Sellards pointed out that the entire syndrome of measles had been reproduced by no single investigator, and, furthermore, that there were contradictory discrepancies between the positive reports of different observers.

Blake and Trask took the matter up in 1921. They decided to

work on monkeys, and to use a material in which clinical evidence gave the best reasons for supposing that the virus would be present, viz., nasopharyngeal washings, and to infect their animals by the route presumed to be the normal one—the respiratory passages. In ten attempts to do this, by the intratracheal injection of 5 to 10 c.c. of the nasopharyngeal washings from patients in the early stage of the disease, they produced an infection having the clinical appearance of measles in eight of the animals. In two of these cases the fluid was passed through Berkefeld N filters, in the others it was unfiltered. The incubation period was from 6 to 10 days; the symptoms were catarrhal conjunctivitis, rash, and leucopænia. The animals recovered. In respect of rhinitis, bronchitis, or the constant presence of pyrexia, the disease was not identical with human measles. Blake and Trask claimed to have carried the infection through six passage generations by the intratracheal injection of tissue emulsions from the infected animals. Recovery from the disease was followed by immunity to re-infection.

Whilst, therefore, there is a respectable body of evidence that the disease may be communicated from man to man, and from man to monkey, both by means of the blood and nasopharyngeal secretions, complete agreement upon this score is lacking. It is evident that if the virus is pathogenic for monkeys the resulting infection is a mild one, which is somewhat surprising in view of the serious results of measles in virgin populations and the coloured aboriginal races.

The attempts which have been made to isolate the virus of measles have been many, but only a few of the results obtained are of any importance. Caronia (1921–28) claimed that by the Noguchi technique he had cultivated small Gram-negative diplococci from the blood, nasopharyngeal secretions, and tissues, in cases of measles, and to have reproduced the disease in rabbits by means of these. He also believed that he had succeeded in inoculating children against measles by the injection of his cultures. Meyer (1926) and Takaki (1926) both attempted to reproduce these findings, but with purely negative results. The former also

attempted to demonstrate the protective action of Caronia's cultures, and in this, too, he failed. Takaki has suggested that where positive protection has resulted it may be due to small quantities of measles blood present in these "cultures." McCartney (1927) likewise failed to obtain any growth in Smith-Noguchi media, and believes that Caronia has been misled by the well-known precipitates and granules which develop in this medium and which have so repeatedly proved a source of difficulty and error to its users.

A second school of workers, of which Tunncliff is the chief representative, believes that the disease is caused by a variety of streptococcus.

The organism of Tunncliff, which was isolated in the first instance from the blood during the eruptive or pre-eruptive stage of the disease, was obtained chiefly in anaerobic cultures made in semi-coagulated horse serum, and in blood-ascitic-dextrose-agar shake cultures. Positive results were obtained in 42 out of 50 cases of measles. The organisms as a rule only appeared after 5 to 15 days' cultivation; they grew somewhat delicately, but most of them became acclimatised to aerobic conditions in the second generation. On blood-agar plates a green colour was produced. glucose and saccharose were fermented, but not as a rule mannite or raffinose. The organisms were insoluble in bile and failed to liquefy gelatine. The same coccus was also isolated from the nasopharynx, conjunctival sac, and discharge from the ear in a number of cases of measles. In films the organism appeared in diplococcal form, or in short chains and staphylococcal clumps; it retains the stain by Gram's method and is stated to pass Berkefeld N filters. Tunncliff noted that at the height of the fever, as Dick and Henry had found for scarlatina, a variety of bacteria are present in the blood. The particular organism of measles was, however, the only one found in the early stages of the disease, and it was not described by the workers on scarlet fever in their extended examination of the anaerobic blood organisms.

The evidence for the ætiological rôle of this organism cannot be said to be strong. It rests upon the demonstration of opsonins and feeble complement-fixing antibodies in the blood of convalescents; upon some inconclusive animal experiments with a few

monkeys and a larger number of rabbits ; skin reactions of the familiar Dick-test type in human subjects ; and the therapeutic preventive use of antiserum.

As regards the presence of antibodies, this does not necessarily indicate any causal relationship between the organisms and measles. If the organisms were present in a mere secondary rôle they would none the less be liable to cause the appearance of antibodies as, for example, is notoriously the case in typhus. The animal experiments, which were made upon monkeys, were four in number, and positive results were claimed in three of these after incubation periods of from four to eight days. In rabbits positive results were also recorded, but the changes do not appear to have been striking. There is a considerable difference of opinion as to the susceptibility of the rabbit to the measles virus, for whilst a number of observers claim that it develops an erythematous eruption and Koplik's spots in a high proportion of cases, Purdy (1925) denies its susceptibility altogether.

With an extracellular toxin prepared from her coccus, Tunnichliff obtained a positive skin reaction, indicating susceptibility, in 50 per cent. of persons having no history of measles ; the toxic substance was neutralised by the serum of human convalescents as well as by that of a goat immunised against the special streptococcus. With killed cultures a skin reaction was obtained, which was positive in persons giving no history of measles, but negative in sufferers from measles after the appearance of the rash and in those giving a previous history of measles. Tunnichliff and Hoyne (1926) also prepared a therapeutic goat antiserum by injections of this diplococcus, which Hoyne and Gasul, as well as the previously named authors, subjected to a clinical prophylactic trial upon infants and other susceptible persons who had been exposed to the measles infection. The results may be summarised —

A. (HOYNE AND GASUL) 48 CASES EXPOSED TO INFECTION

*Treated with serum*

*Not treated with serum*

89

9

*Developed measles*

*Developed measles*

5 (no deaths)

8 (two deaths)

B Tunnichiff and Hoyne also attempted the prophylactic inoculation of 105 other susceptible persons, who had been exposed to measles infection, with doses of 5·0 c.c. of this serum. The results obtained were as follows —

Number of Cases	Period between exposure to Infection and Serum Administration	Number developing Measles
83	1—3 days	1
9	4 days	5
18	5 days, or more	18
42	No serum given	42

All of these subjects were aged one year or more. The very favourable results which we have tabulated are somewhat offset by the results obtained at the same time with eleven infants under one year of age, all of whom had serum after the fourth day and all of whom contracted measles

Others, besides Tunnichiff, have urged the importance of streptococci in this disease. Donges (1928) obtained these organisms in fifteen out of twenty-one blood cultures and was inclined to regard them as of ætiological significance. In his case the organisms were long-chain formers or of the *conglomeratus* type. Ferry and Fisher (1926) announced the cultivation of an aerobic, gram-positive, green-producing streptococcus from the blood in cases of this disease. They claimed that their organisms, which differed from Tunnichiff's by being freely aerobic, produced a soluble specific toxin in broth cultures, which gave a positive skin reaction of the Dick type in persons who gave no history of measles or were suffering from measles in the pre-eruptive stage, but a negative reaction in those who had a definite history of measles as well as in convalescents from the disease. A positive reaction could be rendered negative by a subcutaneous injection of the serum of measles convalescents, and the neutralisation of the toxin by such a serum could also be demonstrated *in vitro*. It has been pointed out by McCartney that the claims put forward by Ferry and Fisher

are by no means substantiated by such actual data as their paper contains. For instance, as proof of the statement that persons with no history of measles give positive skin reactions, they record that out of thirty-five such persons tested, fourteen gave positive skin reactions; *i e*, more than a half gave negative reactions! The only legitimate deduction from this experiment would be in a sense directly opposed to their statement. In other respects also their report is extremely unsatisfactory: they construe a sloughing lesion produced in a rabbit, with a spreading hyperæmia of the skin, as resembling the rash of measles; and from the fact that the pooled serum of several convalescent patients agglutinated two out of four strains of streptococcus, conclude that this is significant in indicating an ætiological relationship of their organism to measles.

. . . . .

Summarising the work which has appeared upon the ætiology of this disease, only that of Tunnichliff offers any serious evidence for the implication of a streptococcus. This work, which appears to be careful throughout and not uncritical, does not carry conviction; for although the organisms described were obtained with a fair degree of regularity and, as far as can be ascertained, were not isolated from other lesions, yet they appear to be anything but a homogeneous group, varying in their characters and often tardy in their appearance in culture media. The virus, from the experiments of Blake and Trask, would appear to be definitely capable of passing Berkefeld N filters, and this Tunnichliff claims her coccus is also capable of doing. The results shown by Tunnichliff for the prophylactic action of her goat antiserum certainly seem quite definite, and in a very small series (1926), in which a comparison was made between the protective action of human convalescent and goat serum, the effect of the two was approximately equal.

Whilst the results recorded by this worker are undoubtedly suggestive they are not sufficiently clear-cut to warrant any immediate conclusions being drawn from them.

**The use of Human Convalescent Serum.**—Another aspect of the study of measles, which has greatly developed in the last

decade, has evolved from the experiments of Nicolle and Conseil (1918), who brought into practice the use of human convalescent serum to protect children known to have been exposed to infection with measles. The matter has been widely taken up, and its efficacy and practicability have been abundantly demonstrated. The blood may be collected from convalescents at any time after the fifth day of apyrexia, and is given in doses of 5 to 10 c.c., according to the age of the child (Park and Freeman, 1926). Serum, citrated plasma, and citrated whole-blood have been used by different workers, but chiefly the first two. It is unnecessary to insist that all precautions as to sterility and a negative Wassermann test must be observed. The activity of the serum is still well marked three months after convalescence, though naturally this is somewhat reduced. There is also evidence that some activity is manifested even by serum obtained from individuals long after an attack of the disease.

The effect produced varies according to the period which has elapsed between exposure and the giving of the protective serum. If this is short, *e.g.*, 1 to 4 days, only a few cases of measles occur, most of the children being protected. The virus in this case fails to develop and the immunity is therefore purely passive, disappearing in a few weeks. If serum be given at a somewhat later date, *e.g.*, between the fifth to the eighth day, the result is a more or less modified attack of measles. Here the virus develops, but its activity is checked by the presence of the serum, and the result may vary from an extremely trivial malaise to a typical attack of measles; the latter, however, is usually mild. In such cases an active immunity results, the conditions being closely similar to those obtaining in virus-antiserum artificial immunisation. It is recommended by some authorities that in healthy children over three years of age, and where the prevalent type of measles is mild, the prophylactic administration of serum should be deliberately postponed until the sixth or seventh day, in order that an active immunity may result. Where serum is given after the onset of the disease its effect, if any, is merely a therapeutic one. In this respect its value is very doubtful. Weaver and Crooks (1924) gave large doses of from 35 to 40 c.c. on the first

day of the eruption, without apparently modifying the course of the disease.

An animal serum has been introduced by Degkwitz (1926) which its author claims is active as an immunising agent in the same way as human serum is. Degkwitz recommends that it should be administered on the fifth day after exposure to the infection, to produce active immunity and a modified attack of the disease. This preparation has unfortunately been patented and put on the German market, and its preparation is shrouded in mystery; it is believed, however, to be prepared from sheep by the inoculation of filtered sputum and nasal mucus from patients. The published accounts of the use of Degkwitz's serum are not encouraging, and it may therefore be said that at the present time the only serum having a proved prophylactic effect is that obtained from human convalescents.

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## TULARÆMIA

Tularæmia is an infective disease of small rodents, such as wild rabbits and ground squirrels, fairly common in certain regions of America and transmissible to man either by direct contact with these animals, as in handling, skinning or cutting them up, or by the intermediary of parasites.

The disease, which takes its name from Tulare County, California, where it was described by McCoy in 1911, would seem to be limited in its present enzootic distribution to America and Japan.

It is a comparatively new disease in human pathology, but since its recognition cases have come to light in increasing numbers. McCoy first described it as a plague-like disease of wild rabbits, and in 1912 McCoy and Chapin found the causal organism in the blood of such animals and designated it *Bacterium tularense*. The first recognised case of human infection was one of ophthalmia, observed by Vail in 1914, from which Wherry and Lamb isolated the organism by infecting guinea-pigs and identified it with *B. tularense*. When the pathogenicity of this organism for man became recognised it was soon shown that the disease known to rabbit dealers as "rabbit fever," and clinically similar conditions known as "Deer-fly fever," "glandular type of tick fever," etc., were also cases of this infection, and the disease was definitely established in nosology. Much credit for this is due to the work of Francis, and Lake and Francis, who in a series of papers dating from 1919 have done much to homologize the variously named clinical conditions due to this infection, and to work out its features as a disease of man. In this country the infection has not been found amongst animals, but human laboratory infections have occurred and were described in detail by Ledingham and Fraser in 1924.

The disease in animals takes the form of an acute and fatal septi-

cæmia associated with enlargement of the lymphatic glands and of the spleen, with the presence of whitish nodular necrotic lesions in this last organ and in the liver; these vary in size from a pinpoint to a considerable nodule and, in the spleen, roughly resemble the appearances of generalised tuberculosis. The transmission from animal to animal is by direct contact and also by the intermediary of rabbit ticks and lice and wood ticks. In the tick the causal organisms remain alive for very long periods, and the infection may be transmitted through the egg to the second generation of ticks.

The organism, *Bacterium tularense*, can be demonstrated in smears, and in sections of the diseased organ stained by Twort's method, often in enormous numbers (Ledingham). The organisms are seen in the liver cells themselves, in macrophages, and free in the capillary spaces. They are minute, gram-negative, non-motile, cocco-bacilli,  $0.7 - 0.8\mu$  in length, and are so small as to pass certain of the coarser bacterial filters. No growth can be obtained on ordinary agar, but growth takes place upon coagulated egg-yolk medium and also upon a special cystine-glucose-serum agar devised by Francis.

The disease in man occurs in two forms :

- (1) As it is most commonly met with amongst dealers and others who handle rabbits and rabbit skins, in a form in which a local lesion is present at the site of entry. This type is also the one previously known as Deer-fly fever, in which the infection is conveyed from animals to man by the bite of a horse-fly. It is not certain whether the organisms can gain entry through the unbroken skin, as some think, or whether a local abrasion is always necessary. In this type of the disease the initial lesion is a necrotic papule, which is followed by acute lymphadenitis of the local group of glands, which become swollen, painful, and in many cases suppurate. There also is considerable fever and general systemic disturbance as in the second type.
- (2) The disease also assumes a typhoidal form, in which no local lesion is to be discovered. This type has been chiefly observed amongst laboratory workers who have

handled infected animals and who contract the disease with great frequency. Ledingham obtained cultures of *B. tularensis* from the United States Hygienic Laboratory, Washington, and during a short period in which the disease was worked at in the Lister Institute three investigators contracted it. It is therefore evident that its infectivity is extremely high. Most American observers believe this to take place through the skin, but Ledingham suggests that in some cases at all events it is air-borne.

In the typhoidal form the disease is of sudden onset, with pyrexia rising to 104° F. or thereabouts and continuing a remitting course for two or three weeks, at the end of which it gradually falls to normal. The glands as a rule are not enlarged, but those of the neck were in Ledingham and Fraser's cases. Convalescence is slow and tedious and marked by short spurts of temperature. Death, in the absence of complications, is rare.

Diagnosis in man is made by the agglutination test. Agglutinins begin to appear during the second week of the disease and are well in evidence at the end of three weeks. The titre is variable, in some of the reported laboratory infections the following results were obtained .—

Case I.	Titre on the 14th day	1 · 640
II.	„ „ 14th day	1 : 160
III.	„ „ 12th „	1 : 80
IV.	„ „ 14th „	1 · 10
V.	„ „ 19th „	1 : 320
VI.	„ „ 21st „	1 : 640
VII.	„ „ 28th „	1 : 1280

The agglutinins seem to continue to rise till late on in the disease, when titres considerably in excess of the above are obtained. They persist for long periods, in four of Francis' cases being from 1 : 40 to 1 : 80 four or six years after the infection was contracted.

Direct culture from the human subject is not a very practicable method of diagnosis, but guinea-pigs are readily infected from the pus of buboes and in some acute cases animal infection has been obtained with the blood. The organism is most satisfactorily

cultivated from the necrotic nodules in the liver and spleen of these animals.

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### Tularæmia

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(A full review of the work of Francis and Lake )

## CHAPTER XII

### RECENT WORK UPON THE PNEUMOCOCCI

THE modern view of the pneumococci dates from the recognition of the serological types, due to Neufeld and Haendel, in Germany, to Dochez and Gillespie, and Cole, in America, and to Lister in South Africa.

Bezangon and Griffin, in 1900, had already noted the presence of agglutinins in the blood of patients suffering from pneumonia and had observed that all pneumococci were not agglutinated by the same serum. The matter was given a more definite turn when Neufeld and Haendel, in 1910, found that the virulent pneumococci from cases of pneumonia with which they worked did not form a homogeneous serological group, but that whilst a large number fell into one class, when judged by these methods, a considerable remnant existed which also differed serologically amongst themselves. They were therefore led to recognise a main group, which they designated "typical," and a number of subsidiary groups which they termed "atypical", these were serologically independent both of the typical group and of each other, but in each of them the members were serologically of the same type. In the light of these facts they considered that serum therapy might be undertaken, and suggested that attention should be first concentrated upon the typical group, since in their experience the organisms belonging to it were responsible for the bulk of cases of pneumonia.

Working at the Hospital of the Rockefeller Institute, in New York, Dochez and Gillespie (1918) studied the serological grouping of pneumococci, using as a preliminary means of differentiation animal protection experiments with sera made against single strains. The results obtained were clear-cut and of the order

shown in the following table, which, as a matter of historical interest, we reproduce here from their original article

CLASSIFICATION OF PNEUMOCOCCI BY PROTECTION  
(62 STRAINS)

	Number	Per cent
Group I . . . . .	28	45
Group II. . . . .	12	20
Group III. ( <i>mucosus</i> ) . . . . .	9	14
Group IV. ( <i>heterogeneous</i> ) . . . . .	13	22
Total typical . . . . .	49	78
Total heterogeneous . . . . .	13	22

These results were obtained by the use of two monovalent sera, prepared from types which preliminary experiments had shown to be the most frequent in lobar pneumonia. Forty out of a total of sixty-two strains fell into one or other of the two groups thus constituted, the first of which apparently corresponded to Neufeld's "typical" variety. Of the remainder, nine were differentiated by their morphology and recognised as belonging to the *P. mucosus* type, whilst thirteen strains failed altogether to fall into this scheme of classification.

The results thus obtained were further confirmed by agglutination, which gave practically identical results to those given by protection tests. Dochez and Gillespie accordingly classified the pneumococci into the now well-known groups. The first two are serological varieties and are generally known as the "fixed types." Type III. is the *pneumococcus mucosus*, whilst Group IV. is a heterogeneous collection of organisms which fail to fit into either of the preceding groups. No notable differences were found in the other properties of the pneumococcus in these different groups, with the exception of the mucoid character of the Type III. strains and a relatively lower degree of virulence for mice amongst the cocci in Group IV.

The grouping set up by the American workers has been very generally accepted, and their results have been widely confirmed. The fixity of Type I. has been universally agreed upon, but the second type is rather less well defined. It has been the experience of many workers that a number of strains of pneumococci agglutinate with Type II serum, but do so rather slowly, and to nothing approaching its titre (Avery, 1915). These have been designated Type II. "atypical" by Stillman (1919), who has extensively investigated these forms and finds a large number of such sub-groups to exist, some of which he has described and named. It would appear probable that the original Type II. of the Rockefeller workers was an organism peculiarly rich in group agglutinogens and that sera prepared from this strain tend to include many organisms which might find themselves in Group IV. if the type were a little more specific. The fact that a number of more recent workers have found the incidence of Type II. in disease to be considerably less than was at first thought to be the case may be due to the increased specificity of their sera. The writer, in the winter of 1924-25, prepared a Type II. serum from an organism isolated from a case of lobar pneumonia and agglutinating sharply with a commercial Type II. serum. This was used by P. Congdon in investigating forty-four cases of severe pneumococcal infection and was found to agglutinate only three of the organisms isolated from these (6.9 per cent.). It may be also noticed, at the same time, that Griffiths, in his analyses of pneumococcal types in pneumonia, found the incidence of this type to be 32.5 per cent in 1920-22, 21 per cent. in 1922-24, and only 7 per cent. in 1924-27. The explanation may be due to an increasing specificity, as we have suggested, or to an actual alteration in the prevalence of the organism. Since it was found by Avery that whilst serum prepared against Type II. (typical) gave protection against the atypical strains, but that sera prepared from Type II. (atypical) did not protect against the typical strains, another explanation may be that these badly agglutinating organisms are of the nature of rough variants which have lost most of their O antigen.

With regard to Group IV, it is recognised that its establishment

is a mere practical convenience, and those who have investigated the matter find that immunological races of pneumococci exist amongst this heterogeneous crowd which are as clear-cut as are the larger group. At the present time, however, these types are so small numerically that their consideration merely leads to confusion.

The importance of the Rockefeller groups has been widely confirmed, and their existence has been reported by Thjøtta and Hanneborg in Scandinavia, Pontano in Italy, and by Thomsen and Christensen in Denmark, in frequency fairly comparable to that found in America and in this country.

In France, largely under the influence of M. Nicolle, a somewhat different view is taken. Nicolle believes that the immunological distinctions between types of pneumococci, or meningococci, founded upon agglutination, are more apparent than real, and result from an inherent lack in such species of the ability to cause marked agglutinin formation. He accordingly teaches that weak agglutinating sera, such as are often in use in dealing with these organisms, are specific, but that more potent antisera have a wider range of action, making the distinction between types less sharp than is maintained by the American workers. From this it is argued that in practical therapeutics highly potent antisera exert a polyvalent effect, although when less active they can only be shown to be effective against the homologous organism. This being so, the agglutination groups lose, for the French workers, any but an academic interest.

Another point of difference which distinguishes the French work is the non-recognition of the American Group IV. This result they achieve by a preliminary treatment of the inagglutinable cocci with dilute hydrochloric acid, which, according to Porges, hydrolyses their capsules and renders them agglutinable. The technique involves the exposure of the organisms to a temperature of 100° C. for five minutes. This procedure is one which, as we have shown in the section upon variation in bacteria, would have the effect of destroying specific agglutinogens, leaving the organisms possessed of group agglutinogens only.

Whilst, therefore, the French view is not comparable with that

taken in this country and in the United States, three pure agglutinable types are nevertheless recognised, which are said to correspond to Types I., II. and III. of Dochez and Gillespie, whilst a fourth named type was a specific one found by Borrel and Kérandel amongst coloured troops in France. Nicolle believes that the more delicate methods of complement fixation indicate the presence of some proportion, at least, of four possible antigenic types in each species of pneumococcus, which therefore form a homogeneous

### LISTER'S RESULTS. 148 CASES OF LOBAR PNEUMONIA

*South Africa, 1913-17.*

Lister's Serological Grouping	Types identified in each Year					American Grouping
	1913	1914	1915	1916	1917	Total
A	9	12	16	4	5	46
B	5	5	1	10	3	24
C	1	3	15	4	9	32
D	3	3	0	0	0	6
E	0	2	0	0	0	2
F	0	0	7	1	1	9
G	0	0	4	6	1	11
Other types = 18				Total		130

race. He takes the view that agglutination specificity is due to the chance predominance of one or other of these common antigenic constituents. This view is one which may in part be brought into line with other work, since the recent antigenic analyses, inaugurated by Weil, Felix, and their co-workers, indicate that in the complement-fixation test a single antigen is concerned (the O antigen), whereas the agglutination test, involving two antigens, allows more room for variation.

Although where the American methods have been followed the fixed types of pneumococci have been constantly recognised, it does not follow that their incidence as causal agents of pneumonia

is everywhere the same, or even that it remains the same in a single locality at different periods. The truth of the first of these statements is indicated by the work of Lister amongst native miners in South Africa. In the years 1918-17 he examined and classified some 148 strains of pneumococci, by agglutination methods, and found them to fall into the several groups shown in the table on p. 252.

It will be seen that the group designated "A" by Lister, which had the greatest incidence throughout the period covered by his investigation, is not recognised in the classification of Dochez and Gillespie, although their predominating types come second and third in order of importance. The identity of Lister's groups B and C with Types II. and I. of the American workers was confirmed by these latter themselves. It may be noted here that French workers (p. 253) also found that the predominating pneumococcus in pneumonia amongst native troops was a fixed serological type, likewise unrecognised by Dochez and Gillespie. The relationship of this type with Lister's type "A" is unknown.

The relative incidence of the different types of pneumococcus in lobar pneumonia, based upon the findings of a large number of workers, is shown in the subjoined table

# INCIDENCE OF PNEUMOCOCCAL TYPES

## I. Lobar Pneumonia

Total Cases	Type I	Type II and subtypes	Type III	Type IV
(a) American Figures				
2144	655	518	221	750
Per cent.	30.4	24.1	10.3	35
(b) British Figures				
524	199	130	21	174
Per cent	38	24.8	4	33.2

(American figures include those of Dochez and Gillespie (1913), Cole (1915), Dochez and Avery (1915), Avery, Chickering, Cole and

Dochez (1917), Clough (1917), Sydenstricker and Sutton (1917), Stallman (1917), Schorer, Clark, Sanderson, Dickson and Hinton (1919), McClellan (1919), Hart (1919), Thomas (1921), Opie, Blake, Small and Rivers (1921)

(English workers. Armstrong (1921), Urquhart (1921), Malloch (1922), Griffiths (1922-24-27), Glynn, Digby and Jones (1923), Congdon and the Author (1925))

## II. Broncho-pneumonia

### *American Workers \**

Total Cases	Type I	Type II and subtypes	Type III	Type IV
879	17	79	91	692
Per cent	1.9	9.0	10.3	78.7

There are not sufficient figures from British sources for a fair comparison of the incidence of types in broncho-pneumonia with that pertaining in America. The general trend of such results as have been published is, however, very similar to these.

The mortality produced by the different types of pneumococcus in lobar pneumococci, as found from a large series of data provided by the authors from which the preceding tables have been taken, is as follows:—

### Mortality

	Type I	Type II and subtypes	Type III	Type IV
Per cent	22.9	35.2	52.8	17.6

It is generally agreed that the mortality of Group III. infections (*pneumococcus mucosus*) is greatly in excess of those due to any of the other types; whilst cases due to Group IV, which includes the bulk of saprophytic pneumococci, have generally a low mortality.

\* Figures taken from Glynn and Digby.

The incidence of the different types in true lobar pneumonia does not appear to be affected by age, being similar over all age groups in the locality in which observations are made. The mortality rates all show a general drop in the earlier years of life in consonance with the well-established clinical fact that lobar pneumonia is a much more benign disease in children.

The very different incidence of the serological types in broncho-pneumonia from that pertaining in the lobar disease is a matter of much interest. Here the most important agents are the organisms belonging to Group IV. of the American classification, which are known to be the common inhabitants of the mouth and pharynx, where the fixed types are much less common under normal conditions. In secondary infections, as for example the post-influenzal broncho-pneumonias which make up the vast bulk of the quoted figures, it is these banal organisms which invade the damaged tissues, and in all probability the same thing could be shown for the broncho-pneumonias of measles or whooping-cough. The investigations of Gaskell, upon experimental pneumonia in rabbits, produced by insufflation, are of interest in this respect. Gaskell found that whilst a pneumococcus of moderate virulence produced discrete broncho-pneumonic lesions, upon intratracheal injection, with organisms of greater virulence more extensive spreading lesions were produced, resulting in a confluent consolidation not unlike that of lobar pneumonia and involving the pleura. It accordingly seems probable, from several considerations, that the type of lesion resulting from the pneumococcal infections of the lungs is very largely determined by the virulence of the invading organisms.

*Infectivity and Epidemiology.*—It has long been known that pneumonia is a disease possessed of a definite, though low, degree of infectivity and prone to undergo quasi-epidemic increases in incidence. Also, that periodic fluctuations in clinical and pathological types occur. It is of some interest to link up these observations with our more recent knowledge of the causal organism.

Acute lobar pneumonia is, in some 60 per cent of cases, due to one of the fixed types of pneumococcus. It has been known,

since the days of Pasteur, that the pneumococcus is with us always, and the explanation of its sudden incursions into the body have usually been founded upon an assumed depression of that nebulous but undoubtedly real property, "bodily resistance"; depressions explained as due to cold, fatigue, alcoholic debauch, etc.—all of which were often found to be lacking.

A study of the incidence of the different types of pneumococci in the normal mouth, made by American workers, has given us the following results (from M. J. Rosenau, 1927).—

Pneumococci absent	Pneumococci present	
42.9 per cent.	57.1 per cent.	<div style="display: inline-block; vertical-align: middle;"> { Type I. 1.8 per cent.  Type II. 5.1    ,,  Type III 8.4    ,,  Type IV 41.8    ,, </div>

It appears therefore that Type I. is hardly present at all, and that Type II. is only rarely present in the mouths of normal persons. A comparison, made in the case of persons in close contact with sufferers from pneumonia, gave the following results —

Type of Pneumococcus in Patient,	Total number of Contacts examined	Number of Positive Contacts (same type of organism as Patient)
I . . . . .	160	21, i e., 13.1 per cent
II. . . . .	149	18, i e., 12.1    ,,

(Monographs of the Rockefeller Institute, No. 7, 1917)

So that the fixed types were very much more frequent in those in contact with the disease than they are in normal persons.

Another study, by the same workers, of the dust of rooms in which pneumonia patients had been lying showed that out of 188 specimens examined, seventy-four contained pneumococci, of which 84 per cent. were Type I., 36 per cent. belonged to Type II. and the subtypes, 3 per cent. were of Type III., and 27 per cent. belonged to Group IV.

It therefore appears clear that the localities in which pneumonia

patients are nursed become infected with the particular types of organisms which are most prone to occasion the disease, and also that persons in contact with patients may come to harbour in their throats virulent types of pneumococci which normally do not exist there.

*Serum Therapy.*—The natural outcome of the recognition of specific immunological strains of pneumococci has been an attempt, by the introduction of monovalent sera, to develop in serum therapy a greater degree of success than had attended its earlier use. The effort has on the whole resulted in some disappointment, for it has been found only possible to prepare a serum of sufficient potency to be of much clinical use in the case of the Type I. organisms. Even here the dosage has to be a large one, 250 c.c. or more, and the treatment has to be early and prompt to be effective. This, indeed, is the greatest obstacle in the way of its successful employment, since as a rule thoughts only turn to serum when the patient is well on in the disease and obviously doing badly. On the whole a number of workers, chiefly in America, have reported well upon the effects of serum, claiming a reduction of the mortality, in the case of Type I. infections, from about 23 per cent. to the vicinity of 12 per cent.

The introduction of monovalent serum has rendered it necessary that the types of the infecting organism should be speedily and accurately determined.

**The Typing of Pneumococci in Disease.**—Various methods are available. Those actually carrying out this work will speedily develop the technique which suits them best. The methods in use fall under two headings :

- (a) Methods for the rapid development of a pure culture of the organism, with its subsequent identification by agglutination.
- (b) Precipitation methods.

The rapid cultivation of the organisms in uncomplicated lobar pneumonia is generally easy, since they are present in the sputum in large numbers and in comparative purity. It can be accomplished best by the method of mouse inoculation, in which a

small mass of sputum, about 0.25 c.c., is washed in several changes of saline, emulsified in broth and injected intraperitoneally into a white mouse. The animal is usually dead or moribund the next morning, and from its peritoneal cavity a few drops of fluid, rich in pneumococci, can be obtained. This is examined microscopically to assure that the organisms are abundant and in a state of comparative purity. The fluid is then allowed to stand, or is briefly centrifuged to throw down blood corpuscles and particles of fibrin, and the pure suspension of pneumococci is then agglutinated against the type sera, used in dilutions known to give a specific reaction only.

In certain cases the simple inoculation of a tube of serum-glucose-broth with a small mass of well-selected and washed sputum will yield a practically pure growth of pneumococci, which can then be centrifuged down and re-suspended in saline for the agglutination test. This is an inferior method to the mouse inoculation one, since it is quite non-selective.

**Precipitation Methods.**—These may depend upon the true precipitin test. Here advantage is taken of the solubility of the pneumococci in bile to obtain a fluid containing precipitable protein, in conjunction with either of the cultural methods mentioned above, or the bile may be used to treat the sputum directly. The fluid is filtered to render it clear and is then mixed with the type sera in dilutions known to be satisfactory; a rapid precipitation occurs with the serum specific for the type of organism present.

In the second case, the precipitin reaction depends upon the specific carbohydrate precipitable substance of the pneumococcus, which diffuses in the body and is present in the urine. A small quantity of the latter is mixed with the three type-sera and incubated for a period up to one hour, at 37° C. The precipitable substance may also be demonstrated in the sputum, by taking a considerable quantity of this, mixing with a little distilled water, boiling to coagulate the albumin, and centrifuging until the supernatant fluid is clear. The thermostable soluble substance can then be tested for with immune sera in the usual manner.

### PNEUMOCOCCAL PERITONITIS

This, occurring as a primary disease and without discoverable pneumococcal infection elsewhere, is essentially a disease of childhood. Its mode of origin has been a matter of some conjecture, the blood, the bowel and the genital tract having been blamed by different authorities. From a survey of the literature, in 1922, McCartney and Fraser found that the disease was one limited to female children and was most prevalent amongst the poorer classes. It usually occurred between the fourth and seventh years of life. McCartney investigated ten cases of primary pneumococcal peritonitis in children, at operation and at post-mortem, and found all of them due to pneumococci of Types I. and II., which were recovered from the pelvis, the blood, *and also from the vagina* in every case. More detailed examination showed the infection to be a spreading one, upwards from the vagina, but that salpingitis, vaginitis and endometritis were not necessary or even usual accompaniments. The possibility of such an occurrence has been previously demonstrated by C. J. Bond, who found that coloured particles introduced into the vagina could later be demonstrated in the peritoneal cavity. In discussing the mechanism of the infection, McCartney points out the frequency with which children of the poorer classes of this age sit about on steps, in doorways and on pavements, which in Scotland at any rate are frequently contaminated with sputum, their vulval region often unprotected by clothes.

### THE SPECIFIC SOLUBLE SUBSTANCE OF THE PNEUMOCOCCUS

This was discovered by Dochez and Avery in 1917, as a result of their investigation of specifically reacting soluble substances found in broth cultures of this organism. It had long been known that such cultures frequently contained soluble products which reacted with specific sera, giving precipitation and complement-fixation reactions. They were generally regarded as being protein substances set free by the autolysis of the organisms, and therefore

similar to the solutions of bacteria, which, as is well known, may be obtained by freezing and thawing and used for the performance of these reactions. Neufeld, in fact, had shown in 1922 that solutions of pneumococci in bile would yield precipitates with immune anti-pneumococcal sera.

Dochez and Avery found, however, that the soluble substance, in the case of pneumococcal cultures, made its appearance within two hours of the time of their inoculation and reached a maximum in about six to eight hours. Now it is known that during about the first twelve hours of growth, under these conditions, the bacteria increase in geometrical progression and little or no death or autolysis can occur. It became evident therefore that the reacting substance was not an autolytic but a secretory product.

In a further investigation of the properties of this substance they found that it was formed in the animal body as well as in cultures, and might be demonstrated in pus and infected fluids, in the blood and urine of infected animals, and in the urine of patients suffering from lobar pneumonia. There was also a rough correlation between the quantity of the substance present in the body and the severity of the infection.

The reaction was found to be a specific one, the materials containing the soluble substance of Type I. pneumococcus yielding a precipitate only with Type I. antiserum, and the same specificity holding for Types II. and III. On the chemical side the substance was found to be thermostable; capable of concentration by evaporation at 100° C.; precipitable by an excess of alcohol, acetone or ether; capable of re-solution in water, and unaffected by protein digestion. Dochez and Avery at this time considered it protein in nature.

As far as can be judged, Dochez and Avery did not at the moment appreciate the full significance of their findings, and they remained as isolated observations until the authors were again stimulated into activity by the observations of Zinsser and Parker who, in 1928, prepared from the filtered alkaline extracts of ground bacterial bodies substances which yielded specific precipitation and fixation reactions with antisera, but which were free from

coagulable protein. They found, further, that such substances could be obtained from influenza bacilli, pneumococci, and meningococci by extraction alone without grinding, and were present in solution in broth cultures of these organisms. An analogy with the substances described by Dochez and Avery was suggested.

In 1928 Heidelberger and Avery returned to the problem and undertook the analysis of their product upon a large scale. They obtained, from some seventy-five litres of culture, about a gramme of a purified material, which had the astonishing composition of only containing 1.2 per cent. of nitrogen and yielding 79 per cent. of reducing sugars on hydrolysis, from which a glucosazone could be prepared. This concentrated preparation gave a precipitate with the specific type-serum when diluted to the extent of  $8 \times 10^{-6}$ .

They therefore concluded that the specific soluble substance was not a protein, as had been at first thought, but a carbohydrate. The small quantity of nitrogenous substance present appeared to be due to an impurity, since the activity of the preparation increased in proportion as the nitrogen content diminished; and the optical activity with the concentration of the specific substance. The material was found to be completely devoid of antigenic powers. Injected into animals it gave rise to no detectable anti-bodies.

It had been known for a considerable time that carbohydrate entered into the composition of bacterial capsular substance, and this had been described as "bacterial gum" by German workers, so that it appeared possible that the soluble substance of Avery and his colleagues might be related to the capsular substance of the organisms.

In later work the chemical composition of this material has been more fully investigated (Heidelberger and Avery, 1924; Heidelberger, Grebel and Avery, 1925), and polysaccharides have been isolated from all three of the fixed pneumococcal types. These have proved to be both chemically distinct as well as type-specific. No claim is made that absolute purity has been reached, but repeated isolations have yielded wonderfully uniform results and the same substances, of identical composition, have been

obtained by different methods of purification. The constitution of these purified products is given in the following table, a single analysis being taken as an example in each case from a large number of very closely similar ones quoted by Avery and his colleagues.

### ANALYSIS OF PNEUMOCOCCUS SPECIFIC SOLUBLE SUBSTANCES

Preparation Number	Specific Rotation	Total N	Amino N.	Reducing Sugars on Hydrolysis	Ash	Dilution reacting with Immune Serum
Type I						
36 . .	+ 304	4.6	2.4	31.6	1.5	1 6,000,000
Type II.						
25 c	+ 72	0.12		67.6	.	1 5,000,000
Type III						
30 a .	- 35.1	0.0		71.0	0.0	1 6,000,000

The well-marked differences between the soluble substances of the different pneumococcal types is here seen. Type I. material contains nitrogen as an essential constituent, but this is absent from the preparations made from Types II. and III., except as an impurity.

The matter has been taken up by other workers and results of a similar nature obtained with many different species of bacteria. Toennissen, in Germany, prepared a nitrogen-free polysaccharide from cultures of Friedlander's bacillus, which yielded an ozazone on hydrolysis; a result which has been confirmed by Avery and his co-workers, who have further shown its specific nature. Mueller and Tomcsik (1924) have isolated a like substance from yeasts, Lancefield (1925) from *streptococcus viridans*; Mueller (1925) from yeast and tubercle bacilli, and Laidlaw and Dudley (1925), in this country, from tubercle bacilli. The last-named workers consider the material to be of the nature of a gum, and

found that it gave a precipitate with an immune serum in a dilution of 1 in 6,000,000. No reaction for glucose was present, but on hydrolysis an unidentified ozazone could be obtained. Landsteiner and Levine (1927) have obtained a similar substance from *V. cholerae*.

For the investigation of the properties of this substance, and the part it plays in the activities of the organism, we are also largely indebted to Heidelberger and Avery. They have made a number of interesting comparative examinations of the properties of the soluble substance with those of the nucleo-protein of pneumococci, prepared by the solution of the organisms in bile and the precipitation of the material with weak acetic acid, or, alternatively, of protein material obtained by autolysis. The two protein preparations gave very similar results.

Whereas the polysaccharide has shown itself to be invariably devoid of antigenic power, the proteins are definitely antigenic but lack type specificity. A serum prepared from one such antigen is active, not only against the homologous protein, and other similar preparations of the same pneumococcus type, but also against pneumococcus proteins in general. Split off from the polysaccharide, the pneumococcus protein, therefore, loses type specificity, and this cannot be restored by mixing the two preparations *in vitro*. For type specificity to exist in the antigen, and to be reflected in the antiserum, the intact organism is necessary. It therefore appears that the type-antigen is a complex of bacterial protein plus the polysaccharide, and that this latter is the essential specific factor. It is worthy of note that although Heidelberger and Avery did not find any development of true type agglutinins in response to the protein antigen, yet they did find that with unheated organisms a fine granular precipitation was sometimes produced by the serum. Since it is now generally assumed that the specific substance is related to the capsule or ectoplasm of the organism, this ill-formed agglutination may have been an effect of somatic agglutination alone (p 55). Viewed in this way their work leads to the postulation of two antigenic factors in the pneumococci: (I.) A type-antigen, in which the polysaccharide is associated with the body protein, and (II.) a species-antigen

present in the bacterial protein split off from this The properties of the two may be summarised as follows :—

<i>Type Antigen</i> (whole organism)	<i>Species Antigen</i> (somatic protein)
Sera give .	Sera .
Type agglutination.	React with the protein of all four groups
Type protection.	Give no precipitation with type soluble substances
Precipitation with the type soluble substance	Do not cause agglutination (? granular, with unheated organisms).
	Give negative, or doubtful, protection.

It will be seen how closely these results correspond with those obtained by Weil, Felix and others in the study of somatic and flagellar antigens.

The importance of these observations is far from being limited to the pneumococcus species, but bears widely upon bacteriology and immunity in general. The question of specificity has always been tacitly regarded as a matter of protein constitution and the configuration of the protein molecules of the antigen, a view recently supported by the work of Dale upon racemised proteins. The discovery of the presence of a carbohydrate moiety in certain antigens, and the demonstration of specificity being dependent upon its linkage with the bacterial protein, is an entirely new conception and throws the investigation of specificity far more open than it appeared to be formerly, since carbohydrates are more manageable substances from the chemical point of view than are the proteins.

Heidelberger, Grebel and Avery have found, in investigating the soluble substance of *B. pneumoniae* (Friedlander), that this is closely related to the carbohydrate isolated by them from the pneumococcus Type II. It appears of considerable significance, therefore, that they also found a close serological relationship between these two organisms; an example of heterogenetic specificity. Whilst the matter is far from proven, it may eventu-

ally be found that the presence of the closely related carbohydrate complex is at the root of this serological kinship ; just as, according to the work of Taniguchi, and of Landsteiner and Simms, the Forssman heterogenetic antibody owes its action to the presence of a specific lipoid group. We may ultimately have to re-orientate our views and to consider specificity less bound up with the intimate structure of the protein molecule than with the presence of loosely attached groups of substances of simpler composition.

There is a suggestion, from the later experiments of Griffiths, to be considered immediately, that the linkage of the specific factor to the basic protein substance, in a group such as the pneumococci, is not in itself a specific matter, but that any one of several possible types of the carbohydrate moiety may be assumed by the undifferentiated racial bacterial protein.

### **THE MUTABILITY OF THE PNEUMOCOCCUS TYPES**

A very important study of this question has recently been made by Griffiths (1928), whose results, if duly confirmed, are likely to be far-reaching.

He noted, in the study of the rough avirulent strains of the pneumococcus, produced by the growth of the organisms in their specific type-antisera and by other methods, that the capacity to revert from the rough to the smooth type was very varied in different individual rough strains. The reversion was accomplished by mouse passage and occurred only upon subcutaneous inoculation. Griffiths, in consequence, studied the local conditions which favoured this transformation and found that it was best accomplished by the injection of a large mass of organisms, obtained by centrifuging down a considerable bulk of broth culture.

It would then appear to have occurred to him that the antigenic constituents missing in the rough strains—in which, as had been previously shown, serological type specificity is lost and specific soluble substance no longer produced in any but the smallest traces—might be restored by a suitable addition to the pabulum. He accordingly tried the effect of injecting dead, virulent pneumococci of the same type (killed by heating at 100° C.) along

with the living R strain, and found that reversion to the S form occurred not only with much greater regularity but took place with small amounts of the living inoculum, which, injected alone, would have been incapable of making any headway.

The next and most striking series of experiments were concerned with providing the developing R strains with accessory pabulum in the form of killed virulent cultures of another type. The result of injecting an attenuated R culture of Type II. into the subcutaneous tissue of a mouse along with a considerable quantity of a virulent culture of Type I. which had been killed by heating for two hours at 60° C. was that in a certain number of instances the animals died of septicæmia, and that the organisms recovered from their tissues were *smooth cultures of Type I.* The possible explanations of such a result are that the heated cultures of Type I. still retained living organisms, a possibility which seems remote and which Griffiths controlled with completely negative results; or that the R, Type II., culture had become transformed into a smooth Type I.

Repeating these experiments with different strains, Griffiths succeeded in obtaining the S form of Type II. from the R form of Type I.; and the S form of Type III. from the R form of both Types I. and II. He has also produced smooth virulent strains of Types I. and II. from a rough strain belonging to Group IV.

The explanation of these remarkable transformations, if they are correct, is probably to be found in the simplified constitution of the R forms, in which the type individuality of the organism becomes lost and its antigenic constitution simplified. Given suitable circumstances, and the multiplying condition, it would seem able again to utilise, or build up as the case may be, the lost antigenic constituents and reappear as the virulent S form. It would also appear, from Griffiths' experiments, that the R form of pneumococci, whatever their derivation, are capable of taking up any one of the three additional antigenic substances known to exist, and that the provision of any one of these determines which type shall emerge. Such results were only obtained in the animal body, never *in vitro*.

The larger question, which is fully discussed by Griffiths, is

the relationship of these modifications to the disease. In the disappearance of the fixed types after an attack of pneumonia, and their replacement by the saprophytic Group IV., he surmises a process of reversion under the influence of immune bodies formed during the attack. This would not be an abrupt process, but one carried out through the intermediate R stage which, as this work has abundantly shown, results from the growth of the normal types in immune serum and may well take place in the body at the stage of the disease in which immunity develops.

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## CHAPTER XIII

### RECENT WORK UPON SPIROCHÆTAL INFECTIONS

THE rather loose nomenclature which has been employed in connection with spiral organisms has been recast by Noguchi, who has reviewed the subject and put forward the following scheme of classification for organisms of this type (Fig. 17).

I. It would appear that the term *Spirochæta* was created by Ehrenberg in 1888 for a large spiral organism met with in waters and differing considerably from those pathogenic species to which this name is now loosely applied ; strictly, therefore, it should not be used for any of the species which infect man.

II. Two other large varieties of such spiral organisms, which inhabit the alimentary canal of shell-fish and also occur in water, sand, and marine products, have been recognised. These are .

(a) *Cristispira* : which show a fin-like structure wound spirally around the length of the body, assuming the aspect of a crista or ridge. The body is septate and a terminal filament is absent.

(b) *Saprospira* . as above, but lacking the crista.

III. **Spironema.** Organisms with a spiral flexible body, the coils being open and about five in number ; a terminal filament is present. Genus includes *S. obermeieri*, *duttoni*, *novyi*, *gallinarum*, *anserina*, *theileri*, *vincenti*, *baccalis*, *refringens*, etc , (N B.—In Bergey's Manual (1926) the term *Borrelia* is used for this genus, instead of spironema as in the previous edition, upon grounds of priority.)

IV. **Treponema.** Very similar to the above and of questionable identity with it, but distinguished as a rule by the greater regularity and constancy of the curves A terminal filament is present. Examples . *Tr. pallidum*, *pertenue*, *macrodentium*, *macrodentium*, *calligyrum*, etc.

V. *Leptospira*. A genus recognised by Noguchi in 1917 (*λεπτός* fine *σπειρα* coil), to include the then newly discovered

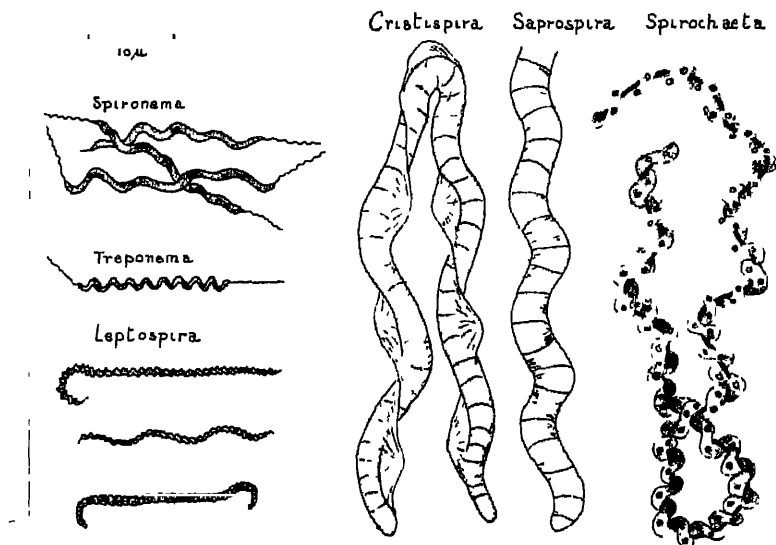


FIG. 17 —Illustrating the structure and proportions of various types of spiral organisms (After Noguchi )

organisms of infectious jaundice. The members of this genus have a few coarse coils or curves, and frequently a hooked extremity.

Organism	Thickness.	Spiral Amplitude	Spiral Depth	Length.
<i>Saprospira grandis</i>	(1) 1.2μ	(7) 8μ	(18) 2μ	(83) 100μ
<i>Cristispira</i>	(1) 1.2μ	(13) 15μ	(5) 6μ	(41) 50μ
<i>Spirochæta obermeieri</i>	(1) 0.5μ	(6) 3μ	(3) 1.5μ	(16) 8μ
<i>Treponema pallidum</i>	(1) 0.3μ	(3) 3μ	(3) 0.9μ	(40) 12μ
<i>Leptospira ictero-hæmorrhagæ</i>	(1) 0.25μ	(2) 0.5μ	(1) 0.25μ	(56) 14μ

The bracketed figures, in italics, give the relative proportions of the various measurements noted, the smallest (thickness) being taken as unity for each genus.

The body of the organism, however, is beset with a large number of minute spirals, which in depth do not exceed the diameter of the organism's body; a distinctive fact. There is no terminal flagellum. Examples: *L. icterohæmorrhagiæ*, *L. icteroides* (vide Fig 17).

The relative dimensions of certain of these types are given on p 270 (Noguchi, *Journal of Experimental Medicine*, 1918, XXVII., 575)

## DISEASES DUE TO SPIRAL ORGANISMS

Five diseases fall to be considered under this head, as having within recent years been attributed to infection with sprial organisms :—

- (1) Rat-bite fever.
- (2) Weil's disease (infective jaundice).
- (3) Seven-day fever of Japan.
- (4) Yellow fever.
- (5) Sand-fly fever.

## RAT-BITE FEVER (SODOKU)

This disease, which is characterised by swelling of the bitten area, long-continued fever, swelling of the lymphatic glands and a maculo-papular rash, was of obscure ætiology until 1916. Up till that time it had been considered, as a result of the work of Schottmuller, as being most probably due to a streptothrix.

There can be no doubt that in the cases described by Schottmuller, as well as in that described by Blake, that streptothrices were present and were a pathogenic agent, if not the sole one. In the light of further knowledge, however, we must regard these cases as not belonging to the typical disease, rat-bite fever, at all, but as being examples of extraneous septicæmias set up by the bite of a rodent, and clinically resembling what is now believed to be a specific disease of known ætiology.

In 1916 a group of Japanese workers, Futaki, Takaki, Taniguchi and Osumi, punctured a swollen lymph gland in a patient suffering from this disease and, on examining the resulting fluid by Burri's method, discovered the presence of spirochætes, which they were further able to demonstrate in sections of the excised gland stained by Levaditi's method. In all (1916-17) the authors described six cases of the disease, in each of which they were able to detect the spirochæte, and in two of them to demonstrate it in the circulating blood. The organisms were at all times scanty in the patient's tissues and were most readily found by inoculating mice, which developed spirochætes in their blood in considerable numbers, after a very variable incubation period which was never less than a week. The infection in mice was not as a rule a fatal one, and the organisms continued to be harboured for several months after it was set up. Both in man and in the mouse the disease is susceptible of treatment by salvarsan.

The spirochæte described by Futaki and his colleagues, as it appeared in films of the blood of patients or inoculated animals,

was a rather short organism whose length varied from about  $2.0\mu$  to  $5\mu$ . It was provided with terminal flagella and stained readily with the ordinary aniline dyes, as well as by Levaditi's silver method in tissues. Large forms were sometimes seen. They named it *Spirochæta morsus muris* and succeeded in cultivating it in Shimamine's medium, where forms as long as  $12-19\mu$  were encountered. Certain observers have described several flagella at either pole of the organism, this, however, is doubtful, and opinion generally tends to group it with the spirilla. The Japanese workers found the organism only occasionally in the rats examined by them, and others have had a similar experience; they did not consider it identical with any of the various spirochætes which at that time had been described in the rat. Both monkeys and guinea-pigs could be infected with this organism, and in the latter animal the infection could be produced experimentally by the bite of a rat which was harbouring the spirochæte. This last observation, on the transmissibility of the infection, had been made previously by Ogata, but without recognition of the part played by a spirochæte in the causation of the disease.

The infection in guinea-pigs was fully studied by Ishiwara, Ohtawara and Tamura (1917), who worked contemporaneously with Futaki and the other Japanese investigators. The symptoms were found to be swelling and congestion of the bitten part, and of the superficial lymphatic glands, with the development of an irregular pyrexia. The animals usually died within a fortnight of being infected. At post-mortem the typical changes were, swelling and congestion of the superficial lymph glands and congestion of the kidneys and suprarenals, with petechial hæmor-



FIG. 18 *S. morsus muris* (After Jeantet)

rhages. They found that rats and mice could carry the organisms without apparently suffering any ill effects, although the spirochætes could be readily demonstrated in their peripheral blood. The guinea-pig, however, always succumbed to inoculation with material from these carriers. They further found that in passage experiments in rats and mice the date of appearance of the spirochætes in the tissues of the animals became progressively earlier, and that they could be found in the peripheral blood four or five days after inoculation, being present in the greatest numbers on about the tenth day. The results of this group of Japanese workers in general conform to those of Futaki and his colleagues, the only point of divergence being the greater pathogenicity which the former found the organism to possess for the guinea-pigs, this, however, may vary with individual strains of the spirochæte. Both sets of observers were able to infect guinea-pigs by the bite of spirochæte-carrying rats, but neither of them demonstrated the organism in the rat's saliva. Salimbeni, Kermorgant and Garcin (1925) found that Chamberland (L. 8) filtrates of the splenic emulsion of infected mice could produce an infection in experimental animals. This observation conforms to a similar one made in the case of leptospiral diseases.

Rat-bite fever is more prevalent in Japan than in other parts of the world, and it is therefore appropriate that we should be indebted to Japanese investigators for its elucidation. Post-mortem examinations of human cases are few, but following upon the publication of the results which we have just recorded, two other Japanese workers, Kaneko and Okuda (1917), had the good fortune to perform post-mortems upon two fatal cases of the disease. They succeeded in demonstrating spirochætes in small numbers in the kidneys, especially in casts and cylindroids in the straight tubules, and also in the supra-renal, and, occasionally, in the testicles. From their studies they concluded that the long and short form of the spirochætes, which had caused some difficulty to other workers, were forms of the same organism. They suggested a distribution of the spirochætes analogous to that of Weil's disease, in that the organisms seem to be present in the blood in the acute stage of the disease, and to appear in the

kidney towards its termination, when immune bodies have developed. The changes found in the cadaver in these examinations were on the whole slight; central necrosis of the liver lobules and fatty degeneration of this organ were the most marked alterations, and were accompanied by parenchymatous changes in the kidneys. In the early stages of the disease the lymphatic glands are swollen and hyperplastic, showing proliferation of the sinus epithelium and erythrophagocytosis, but in the later stages the glandular alterations disappear.

The case for the *Spirochaeta morsus muris* was strengthened by the work of Ido, Wani and Okuda (1917), who demonstrated bacteriolytic antibodies for this organism, both *in vitro* and by means of the Pfeiffer phenomenon, in the blood of patients who had recovered from rat-bite fever. Mooser (1924) has confirmed many of the observations of the Japanese in respect of the characters of the organism as it occurs in rats and in experimentally infected guinea-pigs. He did not obtain any material from human sources. Mooser made the interesting observation that many of the infected animals, both rats and guinea-pigs, develop ophthalmia, and he found the spirochaetes to be present in the conjunctival discharge. He believes that this is the source of infection when the disease is conveyed by biting, which, in view of the failure of all the Japanese workers to demonstrate the spirochaete in the mouth secretions of infected rats, may well be the true explanation of the mode of infection. Manson also mentions the presence of keratitis in rat-bite fever and of spirochaetes in the eye secretions, it would therefore appear that this symptom is a characteristic feature of the experimental infection.

Since the appearance of the original work on this disease confirmation has been forthcoming from widely separated sources. Collier (1924) described two cases in Oxford, in only one of which spirochaetes were found, but both of which reacted to salvarsan. Robertson also described cases in this country. In the United States, Shattuck isolated the organism in a case of the disease, and positive findings have been reported in most of the European countries, as well as from India, Mexico, etc.

The spirochaete would appear to be a far from frequent parasite

of the rat, at all events in Europe. Mlle. Ruys only found it three times in 250 rats in Amsterdam. The organism in this case proved to be pathogenic for guinea-pigs and monkeys; it was not found in the rats' saliva. A similar but non-pathogenic organism was found in mice. Buchanan (p. 286) only found the organism once in 166 Scottish rats which were examined for leptospira. In view of the very considerable and increasing number of cases of the disease now reported, it would appear that the infection must occur in a high proportion of those bitten by rats harbouring the spirochæte.

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## INFECTIVE JAUNDICE (WEIL'S DISEASE)

The causal agent of Weil's disease was unknown until early in 1916, when a paper was published by five Japanese workers—Inada, Ido, Hoki, Kaneko and Ito—giving in considerable detail, and for the first time in English, work published by them in 1914 and the preceding year, in which evidence was adduced that the disease was due to a spirochæte. These investigations were carried out upon a form of epidemic and endemic jaundice which is prevalent in Western Japan and is characterised by jaundice, fever, a tendency to hæmorrhages, albuminuria, muscular pains and conjunctival injection; this was considered to be identical with European Weil's disease.

The Japanese workers succeeded in first demonstrating the organism by the intraperitoneal inoculation of guinea-pigs with the blood of patients, taken in the early stages of the disease, in this way obtaining thirteen positive results from sixteen cases. The disease in the guinea-pigs developed after an incubation period of about seven or eight days, and could be transferred from animal to animal by the inoculation of the heart's blood or the crushed liver. Clinically it showed itself by a rise of temperature and jaundice. The animal dies as a rule within twenty-four hours of the appearance of the jaundice and at post-mortem presents a very characteristic appearance. The tissues are deeply jaundiced and scattered hæmorrhages are seen in various parts of the body, especially in the subperitoneal tissues and in the walls of the intestine. One of the most characteristic appearances is the presence of flecks of hæmorrhage all over the lungs, giving them a similarity which has been noted by repeated writers to the mottling of a butterfly's wing. This finding, together with the icterus, is pathognomonic of the disease. The other organs show acute parenchymatous degeneration.

The spirochætes were first detected in the experimentally

infected guinea-pigs, where they are found most readily in the liver when stained by the original Levaditi method. They can also be demonstrated, though in lesser numbers, but with a fair degree of ease, in the kidneys and adrenals by the same method. The number of spirochætes present in a given case was found by Inada and his associates to be roughly proportionate to the severity of the disease in the animals.

The spirochæte of Weil's disease, which has been classed by Noguchi with the *Leptospira*—a classification which is generally accepted—is known by the ungainly title of *L. icterohæmorrhagiae*. It is a coarse, curved, bent or hooked organism, varying a good deal in size, but being on an average about 6–9 $\mu$  by about 0.25 $\mu$ . It may be as short as 4 $\mu$  or as long as 20 $\mu$ . Noguchi showed that the body of the organism, which in Levaditi preparations looks thread-like, is actually made up of very numerous, closely-set, shallow spirals (*vide p. 270*). There are no terminal flagella. Inada and his colleagues succeeded in getting the organism to pass certain Berkefeld filters; the filtrates were infective for the guinea-pig, but visible spirochætes were not found in them. The cultivation of the organism was successfully achieved in Noguchi's medium, but a temperature of 22° to 25° C. was found much more favourable than 37° C.

In human tissues the spirochæte was detected at post-mortem examination chiefly in the kidneys, where the organisms were found most readily in the tubules and embedded in the substance of renal casts. They were much less numerous in the liver, and, though occasionally detected in other organs, were scanty and often degenerate. It appeared to the Japanese workers that in the later stages of the disease the organisms tended to undergo destruction from the development of immune bodies; they certainly tend to die out, and are more readily found in patients who die early in the disease than in those who succumb later, with a long moribund stage.

The very complete studies of Inada and his associates included some extremely interesting observations upon the route of excretion of the spirochæte and the mode of infection, which throw light upon the way in which the disease is spread under natural

conditions. They found that the organism, in liver emulsion, could penetrate the shaven skin of a guinea-pig when this presented no obvious abrasion. Cleansing the skin with alcohol, or perchloride of mercury, five minutes after the application of the infective material did not prevent the onset of the disease, so that the penetration of the tissues must occur very rapidly. The natural mode of infection had generally, in the absence of any exact knowledge of the causation of the disease, been assumed to be by way of the alimentary tract, and the possibility of this occurring was demonstrated, *inter alia*, in the work we are now considering. The above experiments, however, strongly suggested that a direct percutaneous infection might occur. This idea was strengthened by the clinical observation that in certain Japanese mines, in which the disease was particularly prevalent, the majority of cases were in workers employed below the ground and in certain localities only. Clerks and surface workers largely escaped. The particular groups of miners attacked were those working in wet mines, and in localities in otherwise dry mines in which water had accumulated. When, upon the suggestion of Inada, the water was pumped out, cases of jaundice ceased to occur.

Turning to the fate of the spirochætes in the body, and their mode of excretion, it was found that the organisms disappeared comparatively early in the disease from the circulating blood. The urine, on the other hand, which may be infectious in the early stages, even though spirochætes are sparse, at about the end of the second week comes to contain them in large numbers, especially in the casts and cylindroids. Thereafter they begin to show degenerative changes and ultimately die out, though excretion may continue for as long as forty days after the onset of the disease. Inada and his colleagues suggested that the organisms might live in water and mud and invade the body through the skin of workers who were exposed to contact with these, more especially if they suffered from abrasions of the feet. As a result of the pumping out of water from inundated workings, and ground disinfection, they stated that epidemics had been cut short and the incidence of the disease in epidemic areas greatly reduced. This method of infection has been very generally accepted.

by later workers as the normal one. Experiments upon infection by way of the alimentary tract have given rather conflicting results.

As a result of the findings of the Japanese investigators, a large

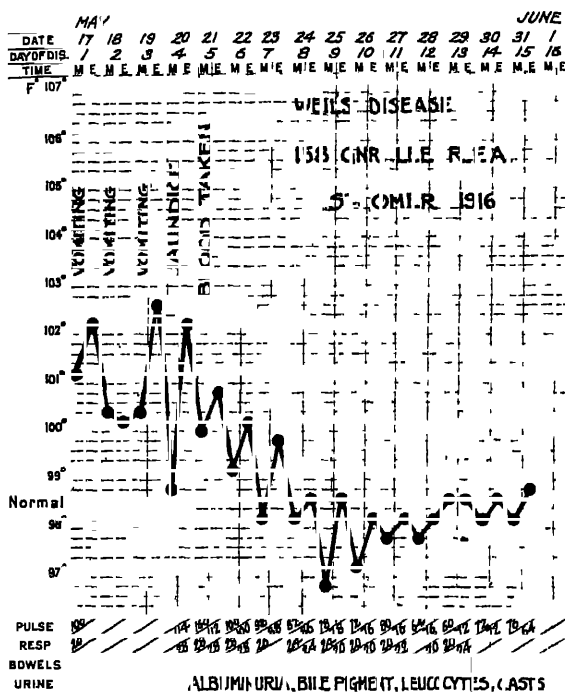


FIG 19—Chart of a case of Infectious Jaundice. The blood taken upon the fifth day of the disease yielded a positive result upon inoculation into a guinea-pig.

number of workers have been interested in this disease, and whilst in many directions our knowledge of the infection has been widened, the initial work of Inada and his collaborators has been very generally confirmed in most of its essential details.

The same group of workers, later in 1916, upon the suggestion of another Japanese, Miyajima, found the Weil's disease spiro-

chæte in the brown rats of city houses, as well as in those of the mines in which they had originally investigated the disease. Spirochætes, indistinguishable from the *L. icterohæmorrhagiæ*, were found by dark-ground examination to be present in the kidney or urine of 82.4 per cent. of the rats examined, and by means of guinea-pig inoculation they were demonstrated in an additional 7 per cent., making the incidence of the organism 89.5 per cent. in a total of eighty-five rats examined. In a later paper, which covered the results obtained with a larger series, the incidence in the brown rat was again found to be 40 per cent. They concluded this work with the significant remark that "the large number of rats in the trenches of the European battlefields suggests the possibility that many cases of Weil's disease may arise."

These words were written in June, 1916, and before they appeared in print the presence of the disease upon the Western front had been established by the forward area bacteriologists, including Stokes, McNee, and the author, and the identity of European Weil's disease with the infectious jaundice of the Japanese workers made certain.

The author's first case of this disease was seen on May 21st, 1916, when he was requested to make a blood examination in what was suspected of being a case of enteric, which had been admitted to a stationary hospital on the previous day. The patient was found to be febrile and deeply jaundiced (Fig. 19), so, in view of the recently reported findings in Japan, the citrated blood was injected into the peritoneal cavity of guinea-pigs. One of these animals developed jaundice eight days later, and was killed on the tenth day, showing at post-mortem the typical appearances. Spirochætes were subsequently demonstrated in the kidneys by Levaditi's method. The great bulk of the infectious jaundice seen upon the British front passed, however, through the hands of Stokes, who, with Ryle and Tytler, worked out the conditions very thoroughly.

Before reviewing the confirmatory work of Stokes and his associates, we may record that two groups of German workers, Uhlenhuth and Fromme (1915), and Hubener and Reiter (1915),

discovered that by the inoculation of guinea-pigs with the blood of patients suffering from Weil's disease a similar condition could be produced in these animals. Both, somewhat later, described spirochætes, the former under the name of *Leptospira icterogenes*, and the latter by the name of *Leptospira nodosa*. Uhlenhuth believes that the contagion can be transmitted by biting flies, but Noguchi failed to find any support for this view. Both of the German workers were doubtless influenced by the investigations of the Japanese, to whom priority must therefore be accorded.

Stokes and his colleagues observed over 100 cases of the disease, and in twenty-six of them were able to infect guinea-pigs from the patient's blood. They were amongst the first to establish the fact that spirochætal infections of this type might lack the characteristic symptom of jaundice, a fact which has been in some danger of being forgotten. In their estimate only about 60 per cent. of the cases investigated showed this symptom. The disease produced in the guinea-pig was as has been already described, and in most cases spirochætes of typical form were found in blood, liver and kidneys. Stokes, Ryle and Tytler also demonstrated the presence of immune bodies in the serum of convalescents and their action in protecting guinea-pigs against injections of virulent liver. They found that most of their cases were admitted to hospital during periods of wet weather, and on tracing their origins found that nearly all the cases occurred amongst troops engaged in the trenches, and more especially amongst those whose trenches were constantly wet and badly drained. Rats caught in these trenches were found in a high proportion of cases to be infected with the spirochæte.

In France, Martin and Pettit, and Monti, upon the Italian front, were also able in their respective countries to confirm the findings put forward by Inada and his colleagues.

A full investigation of the distribution of the spirochætes in the human body has been made by Kaneko and Okuda (1917), who find that this fairly closely corresponds to what pertains in the guinea-pig, and that with the prolongation of the disease and the development of immune bodies, the spirochætes tend to disappear from the tissues. In man, however, they are more scattered and

generally more degenerate in appearance than when seen in the organs of guinea-pigs obtained under more ideal conditions from the point of view of fixation. In the pre-icteric stage of the disease, which lasts up to the sixth or seventh day, the organisms are present in large numbers, especially in the liver, kidneys and suprarenals. Immune bodies begin to be in evidence by about the fifth day, and are associated with a progressive decline in numbers of the organisms. A like effect is brought about by the administration of immune serum. In the icteric stage of the disease the spirochætes disappear from the blood, and its infectivity for the guinea-pig declines very much. The organisms can as a rule no longer be demonstrated in the liver or adrenals, but may still be seen in the kidneys and in the cardiac and other muscles. They are found in the largest numbers in the tubules of the kidney, enveloped in casts. In the convalescent stage, as has already been noted, the organisms are excreted in the urine.

Stokes and Tytler failed to cultivate the spirochæte upon artificial media, probably because they made use solely of aerobic methods. Noguchi (1917) succeeded in growing the organisms from the citrated blood abstracted from the heart of an animal sick of the disease. He recommends two media for this purpose —

- A. Rabbit serum . . . . . 1·5 parts.  
Ringer's solution (or physiological saline) . 4·5 „  
Citrated rabbit plasma . . . . . 0·5 „
- B. A semisolid medium of the same composition, but with 1·0 part of neutral, or slightly alkaline, 2 per cent agar added at 60–65° C., and thoroughly mixed with the fluid.

Both media should be slightly alkaline and should be covered with a thin layer of liquid paraffin. Growth is greatly aided by the presence of small strands of fibrin, which are provided by the citrated plasma. The culture first appears in the zone just below the surface, since the organism requires a certain oxygen tension for its multiplication and fails to grow in solid media into which oxygen does not penetrate.

Noguchi was able to show that Japanese, Belgian and American strains of the organism were identical immunologically and to isolate the same type of spirochæte from the kidneys of American

wild rats. He succeeded in demonstrating numerous morphologically similar organisms in the water of wet mine workings, but in no case did they prove pathogenic. It is obvious from the work of many observers that organisms of the class of *Leptospira* are frequent denizens of water, and they have even been demonstrated in London tap water. It is probable that, as is the case with other bacteria, a large number of essentially different forms exist under the same morphological appearance. Biological and serological methods for the differentiation of these are only just beginning to yield results of any value. Noguchi has utilised resistance to bile and saponin, as well as the ordinary immunity reactions, for the differentiation of spirochætes, and Shiga has suggested the criterion of growth in immune serum. Whilst interesting to record the presence of morphologically similar types in widely differing media, conclusions drawn from their presence must necessarily be guarded. The possibility of decline and acquisition of virulence amongst these organisms seems as possible as it is with the ordinary bacteria, if not more so. Noguchi found that certain of his Guayaquil strains of *L. icteroides* had lost their pathogenicity by the time the cultures reached the Rockefeller Institute, but this was subsequently restored by passage, and Stefanopoulo (1920) found that the lost virulence of cultural strains of *L. icterohæmorrhagiae* could be restored by passage. With abundance of rodent hosts available, and a continued absorption of the organisms from pools of water, such as exist in certain mine workings, and their spasmodic re-pollution by the animal's urine, conditions are set up which might allow free play to this phenomenon. The occurrence of such repeated invasion and excretion of leptospira in the rat is supported by some observations of Buchanan, who in certain rat kidneys which he examined found saprophytic spirochætes of a type common in mine water, side by side with the pathogenic leptospira.

**Bacteriological Diagnosis of the Disease in Man.**—In the early stage of the disease this is best accomplished by the injection of some 3–5·0 c.c. of citrated or defibrinated blood intraperitoneally into the guinea-pig. Up to the end of the fourth day of the disease the chances of this producing a positive result are very high.

Thereafter they diminish rapidly, and after the seventh day are nil. The infected animals succumb as a rule in about five to eight days, and develop jaundice a day or two before death. In the cases in which inoculation late in the disease produces a positive result, the development of the malady in the guinea-pig may be delayed. This is probably a result of the small number of organisms present in the blood at this stage and of the existence of antibodies.

In the later, apyrexial, stages of the disease, and in convalescence, the organisms appear in the urine and can be detected by dark-ground illumination; they are, however, scanty, degenerate, and often far from typical in appearance, and only to be discerned by the centrifugalisation of a considerable amount of urine. Moreover, they rarely infect guinea-pigs. Their impotence at this stage is ascribed by most workers to the destructive effect of bile, acting in a highly acid medium. *L'aute de mieux*, it may be worth while attempting guinea-pig inoculation even now, as success is possible, although unlikely. In cases in which cultures of *L. icterohæmorrhagæ* are to hand a diagnosis can often be made in retrospect by the demonstration of immune bodies, protective for guinea-pigs, in the serum of a convalescent.

**Weil's Disease in Great Britain.**—A few sporadic cases of the disease, of proved or probable spirochætal origin, have been reported in Great Britain, which are in consonance with similar findings in Germany, France and South America. A considerable epidemic of the disease was studied between 1923-27 by Gulland and Buchanan in Scottish mines. In 1924, in consequence of its prevalence, the Scottish Board of Health made spirochætal jaundice a notifiable disease, and arrangements were made for Buchanan to have access to all notified cases. As a result this worker was able to investigate fifty-one cases of jaundice, twenty-two of which he concluded were certainly of the infective type. Slightly under a half of the patients were mine workers, the others came largely from rat-infested areas, such as breweries, piggeries, refuse dumps, etc. The bacteriological proof was not forthcoming in all of these cases, clinical criteria being relied upon in some of them, but the

high incidence of hæmorrhages and the severe nature of the condition makes it probable that the diagnosis was correct. Five of the cases ended fatally. Buchanan succeeded in cultivating the organism in Noguchi's semisolid medium "B" (p. 288) from the tissues of infected animals. In investigating the spread of the infection he found that the organism might survive in moist mine ground soil for as long as ninety-five days; and he actually succeeded in demonstrating the spirochæte in certain slimy material found upon the roof of workings which were markedly damp, and in proving its pathogenicity by the successful direct infection of guinea-pigs. With regard to the presence of the organism in rodents, the same author examined 166 rats and found 36·7 per cent. of them infected with spirochætes.

Such observations, like many others, go therefore to show that the infection is widely disseminated, and is pre-eminently one in which the general high standard of hygiene in civilised countries forms an effective barrier to its spread. As an instance of the curious ways in which such a barrier may fail, we would quote the interesting case reported by Manson Bahr of a sailor who fell into the Thames. His rescue was effected, but was rapidly followed by an attack of typical icterohæmorrhagic spirochætosis.

**Vaccination and Serum Therapy.**—We have already noted the development of bacteriolytic antibodies in the serum of patients in the later stages of the disease, and in convalescents. Such sera have been found to exert a protective action upon guinea-pigs. Inada and his co-workers prepared an antiserum by the injection of horses and used it in the treatment of the disease, as did also Martin and Pettit in France during the war. A. S. Griffiths, in this country, prepared such a serum in the same period, and use was made of it amongst the troops. All of these sera were found to exert a powerful protective action against the spirochæte, in experiments carried out upon guinea-pigs, but their therapeutic action was much less marked. The Wellcome Research Laboratories prepared a serum from the Scottish strain of leptospira, isolated by Buchanan, and the members of that institute, to whom the research into "yellows" in dogs is due (*q.v.*), found it to have

both a prophylactic and curative effect in this disease. One of their observations, which bears upon the time factor in administration, is worth quoting. Dogs were injected intraperitoneally with infected liver and the serum given at varying intervals thereafter. The results are summarised below.

Number of Animals	Quantity of Serum Given	Interval between Infection and the Administration of Serum	Result
	cc	Hours	
2	10·0	24	Lived.
2	10·0	48	Lived.
2	10·0	72	Lived.
4	10·0	96	Died.
1	<i>Nil.</i>	—	Died.

Since in the experimentally infected dog jaundice does not, as a rule, occur before the fifth or sixth day, it is evident that the serum loses its power before the classical symptoms develop. Nevertheless, in the spontaneously occurring disease, these workers consider that the results of serum therapy have been encouraging.

The results of clinical experience in man are in pretty close accord with such experimental findings, with which all workers are in agreement. Although the serum has an actively bacteriolytic effect, its use, at the stage in the disease when jaundice has become apparent, is not productive of any very striking beneficial results. At this stage the spirochæte has already become scanty in the organs, with the exception of the kidney, where the serum does not seem to affect it much. Histological study of the injury produced by the disease in liver and kidney reveals that at this stage the patient's functions are already gravely damaged and there is, therefore, not much to be hoped from the serum if its action is solely a destructive one upon the spirochæte. The matter is altogether different in the earlier stages of the disease, but unfortunately it has not been usual, nor

is it at all easy, for a diagnosis to be made at this period, in which most is to be hoped for from its use.

Prophylactic vaccination was undertaken by the Japanese workers, in the first place in guinea-pigs which were injected with killed emulsions of infected liver and, later, with phenolised cultures of the spirochæte. It was proved thereby that active

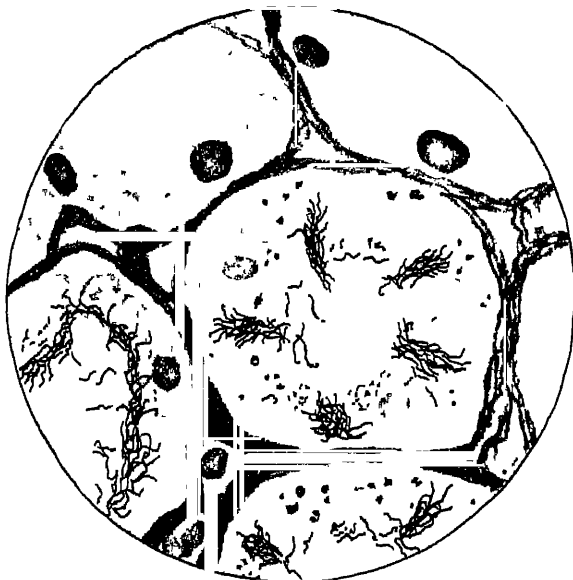


FIG. 20 —*L. icterohæmorrhagæ* in the kidneys of a wild rat.  
× 1000. (After Buchanan.)

immunity against the experimental disease could be established. The method was then extended to man, and good results were claimed for it; it certainly seems worthy of trial in cases where the disease is epidemic. Dalling and Okell (1926) immunised dogs, exposed to leptospiral infection, with phenolised liver emulsions from infected guinea-pigs, and apparently produced a satisfactory immunity.

**Leptospiral Infections in Animals.**—The rat is the normal reservoir of these pathogenic leptospira. Mice have also been

found infected, and Ch. Nicolle and Lebailly have shown that after being artificially infected this animal can harbour the parasite for very long periods and may, in fact, be used as a means of preservation of the spirochæte. All epidemiological evidence points, however, to the superior importance of the rat as an animal vector of the infection. In various parts of the world a high incidence of the infection in the common brown rat has been observed, often in the vicinity of 30 to 40 per cent., and existent both in sites in which the disease has been observed and in those in which it is normally absent (*e.g.*, London). The organisms appear to live in comfortable symbiosis in the rat, where they are concentrated in the kidneys, appearing in, between, and on the surface of the tubule cells (Fig 20). A combination of an abundance of surface water and slime, in which human subjects soil themselves, defective hygienic conditions, and numerous infected rats are the requisites for an outbreak of the disease. Sporadic cases may be due to direct soiling of food by rats.

In dogs, Okell, Dalling and Pugh (1925) discovered a spirochæte in the spontaneous disease known to kennel-men and veterinarians as "the yellows." It had been shown in 1917, by Courmont and Durand, that the dog was susceptible to the spirochæte of Weil's disease, either by inoculation or by ingestion, and that in the case of young animals an infectious febrile jaundice, terminating fatally, often developed. Okell, Dalling and Pugh succeeded in isolating an organism, morphologically indistinguishable from the *L. icterohæmorrhagæ*, in three out of ten cases of enzootic canine jaundice which they were able to investigate. The organisms were found in fresh preparations of the animals' tissues examined by dark-ground illumination, and the ground-up liver produced typical spirochætal jaundice when injected into guinea-pigs. Protection against the organism was provided both by anti-icterohæmorrhagiæ serum, as well as by the serum of a dog which had recovered from the disease. Puppies experimentally infected with *L. icterohæmorrhagæ* contracted a fatal disease in all points closely resembling the spontaneously occurring "yellows."

The results of the English workers to some extent have been

confirmed by Klarenbeek (1927), in Holland, who was able to find spirochætes similar to those of Weil's disease in the blood of jaundiced dogs during life, and after death in the kidney stained by silver impregnation. In this organ the spirochætes were distributed chiefly in the interstitial tissue and occasionally in the tubular epithelium. This worker, unlike the English bacteriologists, did not succeed in obtaining any positive results by animal passage. It may be remarked, however, that most of his cases were seen late on in the disease and that the series examined was a small one.

There also occurs in dogs a somewhat obscure epizootic disease known as canine typhus or "*maladie de Stuttgart*," in which Lukes (1924) described the presence of spirochætes. The disease is characterised by thirst, foul breath, vomiting, diarrhœa with blood in the fæces, low temperature and somnolence. The urine is of low specific gravity and contains albumin and casts. The blood urea is raised. At post-mortem a hæmorrhagic gastro-enteritis has been found, together with necrotic ulcerative lesions of the buccal mucosa. The cause of death would appear to be uræmia. Renal lesions are always present, and in these organs Lukes described the presence of numerous spirochætes.

Klarenbeek, in fifteen cases of this disease, has likewise demonstrated spirochætes, using the original silver impregnation method of Levaditi. They occur in large numbers, chiefly in the straight tubules of the renal papillæ, and less often in the secreting tubules. They are rare in the interstitial tissue. The spirochætes are massed on the surface of the lining epithelium, and appear more or less free in the lumen of the tubules. The organisms show a tendency to break up, and the silver preparations show a multitude of small granules which are attributed to their fragmentation.

All attempts at the experimental transmission of the disease have failed, and the intraperitoneal injection of the infected tissues in the rabbit and guinea-pig have given negative results. Although there is scope for further experimental investigation in this direction, we may say in passing that the strict localisation of the spirochæte to the kidney and its appearance at the surface of the epithelium, which so closely recalls the picture given by the

*L. icterohæmorrhagiæ* in the rat (Fig. 20), suggest that the organism is present rather as a relatively harmless saprophyte than as the ætiological agent of the disease.

Some curious observations by Wilbert and Delorme (1927) have been reported from the Pasteur Institute of Kindia, French Guinea. An outbreak of acute febrile jaundice occurred amongst the chimpanzees, of which a large colony was kept at this institute. The disease originated in a batch of animals recently imported from the Ivory Coast, and eventually the whole stock at Kindia, twenty-four animals in all, were attacked and twenty-three died of the disease. Upon histological examination a spirochæte resembling the organism of spirochætal jaundice was found in blood, liver, spleen, kidneys and central nervous system. The results of experimental inoculations showed that whereas the chimpanzee contracted the disease readily and in a fatal form, the rabbit, mouse and dog appeared refractory to the infection. In the case of the guinea-pig the inoculation resulted in a febrile reaction, which was not as a rule fatal, but which could be transmitted from guinea-pig to guinea-pig in series. All of these animals showed the presence of spirochætes in the blood for some days, and an occasional one developed icteric changes and died. In the course of the investigations Wilbert contracted the disease himself, suffering from fever, slight icterus, and the vomiting of bilious material streaked with blood. A specimen of his blood, taken on the fourth day of the disease, was injected into a chimpanzee, which contracted a fatal form of the illness. The authors designate this condition "spirochétose ictérohemorragique," although it does not show the features of Weil's disease as familiar to most workers. In many ways there is greater similarity with the results reported by Noguchi in yellow fever, and the authors discuss this point and remark that the animals were brought from an area in which the latter disease is endemic. They also discuss the spirochætosis of the rats which abounded in their animal houses, and conclude that the leptospira in these could be differentiated from the ones found in the infected chimpanzees. The recent work of Stokes, Bauer and Hudson, in West

Africa, has shown that the chimpanzee is resistant to the virus of yellow fever, so that the findings of Wilbert and Delorme throw an interesting sidelight on the whole question of the presence of leptospira in this disease, presently to be discussed, and Noguchi's findings.

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## SEVEN-DAY FEVER

This is a malady met with in certain parts of Japan, which is characterised by sudden onset, with malaise, temperature, conjunctival congestion, myalgia, digestive disturbance and enlargement of the lymphatic glands. The disease runs a short course, and is non-fatal. It occurs chiefly in country districts.

For some time prior to the discovery of the causal organism of Weil's disease the identity of this seven-day fever with that complaint was a matter of discussion, though Inada, who made a careful comparative study of the two diseases, concluded that they were distinct.

In 1918, following upon the demonstration of the leptospira of Weil's disease, Ido, Ito and Wani, who had all taken an active part in the work upon the latter, announced that a spirochæte was also to be found in seven-day fever. This organism they called *Spirochæta hebdomadis*, and differentiated it from the infectious jaundice organism. Subsequently it has been classed with the *Leptospira* in Noguchi's nomenclature, under the name of *L. hebdomadis*.

The method of investigation followed was essentially that employed by the previous workers upon Weil's disease. Guinea-pigs were inoculated with the blood of patients, they developed fever, and in some cases died. In the animals which succumbed were lesions which resembled those produced by the Weil's disease spirochæte, but were definitely less in degree. Jaundice was slight, hæmorrhages were less marked, and swelling of the lymphatic glands was a prominent feature. Spirochætes could be demonstrated in the blood of these animals, and after death in the liver and kidneys. The infection was not one which was easily propagated from animal to animal in series, as certain animals, especially the older ones, resisted, and the infection consequently tended to die out.

The spirochæte, which on rare occasions could be demonstrated in the blood of patients, resembled *L. icterohæmorrhagiæ* in appearance, but could be distinguished from it by the Pfeiffer test and by the absence of any reciprocal protection conferred by the serum of animals which had survived one or the other infection. Animals which recovered from infection with the seven-day fever virus were immune to further inoculation, but were susceptible to inoculation with *L. icterohæmorrhagiæ*, and *vice versâ*. At the same time there is in this infection a much less marked tendency to jaundice production than is the case with either *L. icterohæmorrhagiæ* or *L. icteroides*. Rabbits were to some extent susceptible to the disease. The spirochæte was present in the urine of patients, especially during the period of convalescence, but it was only exceptionally that it retained its pathogenicity for guinea-pigs in this situation.

Still following the lines of investigation which proved so fruitful in Weil's disease, Ido, Ito and Wani cast about for a rodent host for the parasite, and found this in the field-mouse (*Microtus montebelli*). The organism was found in 3 per cent of these animals, localised in the kidney and present in the urine. The disease was also found to be prevalent in districts in which these animals abounded.

Recently Koshina, Shiozawa and Kitayama have investigated another Japanese disease known as "autumn fever," and from it have isolated spirochætes also resembling *L. icterohæmorrhagiæ*. They distinguish two serological types. One of these, type B, is identical with *L. hebdomadis*; the other, type A, is more virulent and does not correspond serologically to either *L. icterohæmorrhagiæ* or *L. hebdomadis*, although, like the latter, it is harboured by the field-mouse.

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## CHAPTER XIV

### RECENT WORK UPON SPIROCHÆTAL INFECTIONS (*contd.*)

#### YELLOW FEVER

"THE germ of yellow fever is not a bacterium, at all events it is not a visible bacterium . . . it cannot be cultivated or modified on the ordinary bacteriological lines" (Manson, 1914).

The position with regard to yellow fever in 1917 was that stated by Manson. The careful work of Sternberg had disposed of the various bacterial claimants which had been put forward as the cause of the disease, and that of Marchoux and Simond had shown the virus to be capable of passing Chamberland F filters, but not the finer-grade, B, candles. It will likewise pass Berkefeld V and N filters, but is held back by the less porous, W candle.

The germ was known to be present in the blood of patients during the first three or four days of the disease only, and the natural agent for the transmission of the malady was recognised to be the *stegomyia* mosquito. The fact that the mosquito only became infective twelve days after its meal of blood pointed strongly to the causal organism's undergoing a developmental cycle in the body of the mosquito. On the side of animal experiments, Thomas claimed to have transmitted the disease, in a single instance, to the chimpanzee through the medium of mosquitoes, and possibly also to the guinea-pig.

There was, therefore, a mass of accumulated knowledge about yellow fever, and a great deal of inferential information concerning the properties of the virus, although this had never itself been demonstrated.

In the year 1919 and the following years, a series of papers appeared at the hand of Noguchi, claiming to have demonstrated that the causal agent of the disease was a spirochæte, of the type

leptospira, closely allied to the *Leptospira icterohæmorrhagiae*, but distinguishable from it.

Noguchi, as a member of the Yellow Fever Commission of the American International Health Board of the Rockefeller Foundation, worked at Guayaquil, Ecuador, where he had access to an abundant material in the shape of 172 cases of yellow fever. In June, 1919, he announced that as a result of many attempts to convey the disease to different mammals, including ringtail monkeys and birds, he had obtained positive results in 8 out of a total of 74 guinea-pigs, this representing material from 6 cases of the disease out of a total of 27 cases examined in this way. The infected guinea-pigs, after an incubation period of from 3 to 6 days, showed fever and later jaundice, and sometimes, though not always, succumbed to the malady. In the case of an animal dying of the disease Noguchi succeeded in demonstrating leptospira in emulsions of the kidneys and liver, but not in the blood. This organism he has named *L. icteroides* and regards as the causal agent of yellow fever.\* Retrospective investigation of previous work has since brought to light the fact that Stimson, in 1909, discovered a similar organism in the kidney of a case of yellow fever stained by Levaditi's method. This organism, which appears to be identical with the *L. icteroides* of Noguchi, was named *S. interrogans* by Stimson, who was not clear if it was really a spirochæte or what its significance might be. It may also be noted that Seidelin believed that he had shown the possibility of infecting guinea-pigs from patients suffering from yellow fever. He, however, was led into interpreting certain bodies seen in blood cells as being the parasite, a view which has been generally rejected.

In later papers Noguchi developed this work. He found that on an average the incubation period in guinea-pigs was about six days. The blood of the patient only exceptionally produced a decided positive result in these animals, although many of them, which showed no reaction or at the most an

\* Whilst this article is in the press, news has come of the regretted death of the able Japanese scientist, from the malady against which he was waging his latest fight. His name goes to be added to the long list of investigators who have succumbed to this very fatal disease.

indefinite one, were subsequently immune to inoculation with cultures of the leptospira which killed the controls. In the case of animals reacting positively the infection could be transmitted to further guinea-pigs by the inoculation of the tissues of the first and the infection thus carried on in series. The organisms were found to be constantly present in animals which developed the disease and could be demonstrated in their blood, liver and kidneys. Cultures of leptospira were obtained from the infected animals and were found to be pathogenic for other guinea-pigs, as well as for dogs and marmoset monkeys, though not for the South American ringtailed monkeys.

Applying these results directly to the human subject, Noguchi succeeded in obtaining positive cultures from the blood of patients in three out of eleven cases and in detecting the organism in the peripheral blood stream, in very small numbers, in three out of twenty cases. Only once was it discovered in an organ after death, and on that occasion in the liver. Noguchi explains this by the statement that the organisms die out and disappear from the tissues in the moribund phase of the disease as well as after the death of the patient. In contrast to what pertains in infective jaundice, the spirochæte is not detectable in the urine.

The general morphological features of the organism closely resemble those of *L. icterohæmorrhagica*, to which it is very similar in other respects. In culture, according to Noguchi, the organism thrives best in semi-solid media, such as serum diluted with three parts of Ringer's solution and stiffened by the addition of 0.3 per cent. of agar. It grows best under conditions of partial anaerobiosis, in this respect also resembling the organism of Weil's disease. Guinea-pigs showed themselves highly susceptible to cultures of the spirochæte and often succumbed to extremely small doses, although the animals showed a remarkable range of individual variation in this respect. Noguchi found that the organism grew well at 25-26° C. and was capable of passing Berkefeld V and N filters, it would therefore be capable of pullulating within the body of an insect at ordinary tropical temperatures, and possess that filtrability which had long been known to be a property of the yellow fever virus.

The filtrates of leptospiral cultures, although infective, did not show any visible organisms, an observation which raises afresh the question of the existence of a granular infective phase in the life-history of the spirochætes. This Leishman believed he had observed in the organism of relapsing fever, and Balfour also upheld its existence in the spirochætosis of fowls.

In respect of the further agreement of the characters of this organism with the previously determined facts about the virus of yellow fever, Noguchi claimed to have successfully infected one guinea-pig out of six by the bites of *stegomyia* mosquitoes which had been previously fed upon a patient suffering from yellow fever. He also found that upon two occasions he was able to transmit the disease from guinea-pig to guinea-pig by means of the mosquito. In this connection the observation was made that the latent period of twelve days, which normally lapses before the insect becomes infective, was shortened in the case of the guinea-pig disease to eight days. What happens to the virus in the body of the mosquito has long been an unsolved mystery, and from the known facts of malaria a cycle of development was freely inferred for the yellow fever parasite. Noguchi was able occasionally to find leptospira in the crushed bodies of mosquitoes, by dark-ground examination, and suggests that the shortened latent period in the case of the guinea-pigs resulted from the heavier infection current in these animals. From this point of view a simple pullulation of the organism is all that might be supposed to occur in the mosquito's body, and the latent non-infective phase would merely represent the time required for the leptospira to reach an effective concentration.

Noguchi also investigated the serological reactions of the spirochæte and its relationship to *L. icterohaemorrhagiae*. He found further support for his belief that it is the causal agent of the disease in the fact that the serum of convalescents from yellow fever gave a positive Pfeiffer reaction with cultures of the organism, and also, in some cases, conferred passive immunity upon guinea-pigs against inoculation with cultures. With regard to its relationship to the spirochæte of infectious jaundice, he found that a monovalent icteroides serum agglutinated all his strains of

the Guayaquil organism but gave little or no agglutination with the Weil's disease spirochæte, and that the converse also held good. In protection experiments upon guinea-pigs, using polyvalent icteroides and icterohæmorrhagæ sera, he found a good deal of overlapping but claimed that when very careful adjustments were made a sharp differentiation between the two types could be observed. Complement fixation tests gave similar results. Differences were also noted in the pathological effects produced in the experimental animals, the Weil's disease spirochæte showing a greater tendency to produce hæmorrhages than the *L. icteroides*; the latter tended to produce more marked nephritic and parenchymatous lesions, with fatty degeneration of the liver and hæmorrhages in the gastric mucosa.

Noguchi discussed whether this evidence should be taken to indicate specific difference or merely strain variations within a single genus; he concluded, rather guardedly, that the two organisms were closely allied but nevertheless distinct races. He thought, as an outcome of his work, the serum treatment of yellow fever to be within the range of practical therapeutics, since in the incubation and early stages of the malady in animals it had proved both preventive and curative. He has produced an immune horse serum for this purpose, 1.0 c.c. of which will neutralise 5,000,000 minimum lethal guinea-pig doses of the spirochæte.

In further studies of the disease in Mérida, Yucatan, and in Northern Peru, Kliger and Noguchi obtained similar results to those in Guayaquil. In Vera Cruz, Perez-Grovas (1921) claimed to have transmitted yellow fever to guinea-pigs and to have obtained cultures of *L. icteroides* both from the blood of these animals and directly from patients.

Noguchi, with Muller, Torres, Silva, Martins and others (1924), also carried out similar investigations upon the yellow fever which, in spite of prophylactic measures, still exists in certain parts of Brazil. They succeeded in isolating the *L. icteroides* on two occasions by direct blood cultures taken in five cases of the disease. The organisms when first isolated were of feeble pathogenicity for guinea-pigs, but this increased considerably upon

passage until its virulence for these animals became high; the lesions produced being the usual ones of jaundice, lung hæmorrhages, hæmorrhages into the stomach, nephritis and fatty degeneration of the liver. The demonstration of the leptospira was difficult. Two local monkeys (*cebus macrocephalus*) were inoculated with passage strain of the spirochæte and developed the disease. One was treated with an anti-icteroides horse serum, prepared from the Guayaquil, Peruvian and Mexican strains of this organism, and recovered, the other was not treated and succumbed to the disease, presenting post-mortem findings similar to those of yellow fever in the human subject. No leptospira were at any period or by any method demonstrable in its body. Cultures of the Brazilian strains were also found to be pathogenic for puppies, and in other respects to present characters similar to those already described for the spirochætes in yellow fever in other areas. The sera of Brazilian convalescents yielded a positive Pfeiffer phenomenon when tested against the local strains, as they also did against strains from other regions. The reaction was negative with *L. icterohæmorrhagix*. Further, serum artificially prepared from the alien strains powerfully protected guinea-pigs against the Brazilian leptospira.

Preventive vaccination was attempted by Pareja and Noguchi (1921) in Ecuador, and subsequently its use was extended to many districts in South America in which the disease is present. Killed cultures of the organism were used, and two injections given: the immunity takes ten to fifteen days to develop. The reported results are excellent, and the preventive value of the method in this area would seem proven. In a period of intense epidemic activity in Peru, J. H. White inoculated twenty-five out of fifty non-immune soldiers who were quartered in a village in a highly infected district, out of the twenty-five uninoculated men twenty contracted the disease, whilst all of the inoculated escaped. Larger scale statistics, giving the incidence of the disease amongst the inoculated and uninoculated in considerable populations, are likewise favourable to the method. The duration of the protection is uncertain, but from the permanence of the immunity conferred by an attack of the disease it is probably of

considerable length. Of the results of serum therapy much cannot be said at the present moment, except that to be of any use serum must be administered early, and that in such cases its reported results are encouraging.

The results obtained by Noguchi and his collaborators have been given here in their entirety, for the sake of completeness and ease of reference, and without being split up by a consideration of the criticisms which have been passed upon them. These are weighty and are considered in detail on p. 807.

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(See also references under the general discussion on Leptospiroses, p 315)

## SAND-FLY FEVER, DENGUE, PAPPATACI FEVER AND FEBRILE SPIROCHÆTOSES

The clinical similarity between the short, non-fatal, types of fever known as dengue and sand-fly fever, and others to which various local names are applied, is great, and their classification upon clinical lines avowedly unsatisfactory. The inter-relationship of these various forms is likely to remain obscure until their ætiological agents have been discovered and compared

In the case of two such conditions, the "dengue" prevalent in Beyrout, Syria, and the "sand-fly fever" of Malta, Couvy, and Whittingham respectively have described the presence of spirochætes

Couvy noted the organisms in 1921 in the blood of his cases when this was examined two to three hours *before* the onset of the fever. In the year following he was able to repeat these observations, during the currency of a second epidemic, and was again able to detect spirochætes in the hours preceding the onset of fever and also during the first forty-eight hours of its existence. The blood of patients, when injected into rabbits, produced fever in these animals and spirochætes could be demonstrated in their blood. During the epidemic *Culex* and *Stegomyia* mosquitoes were present in the infected area as well as the *phlebotomus pappataci*, but in a similar severe epidemic at Lebanon only phlebotomous flies were found. In two instances the intraperitoneal injection of crushed phlebotomous flies into rabbits gave rise to febrile reactions with the presence of spirochætes in the blood. It would consequently appear, from such evidence, that the disease in question was conveyed by this organism and not by the *Stegomyia*, which is the insect vector of dengue as generally described.

In the case of phlebotomous fever the infectivity of the blood for human volunteers had been already shown by an Austrian Commission (Doer and others), and also by Birt, and it was known

that the infection was demonstrable only during about the first twenty-four hours of the disease. The *phlebotomus pappataci* was recognised as the vector of the disease and the virus had been found capable of passing a Chamberland F. (L. 5) filter. In 1921 Whittingham claimed to have succeeded in cultivating a spirochæte, in a modified Noguchi medium, directly from the blood in six cases out of a total of twenty-six patients upon whom this was attempted. Three of the strains were successfully propagated in subculture. The organisms so isolated were morphologically indistinguishable from *L. icterohæmorrhagæ* and grew best at a slightly reduced oxygen tension as this organism does.

Whittingham also inoculated guinea-pigs with the citrated whole blood of patients suffering from the disease, and found that some of them developed a febrile reaction, whilst animals inoculated with healthy blood did not show this. His experiments were, however, not very satisfactory owing to difficulties with the animals and intercurrent infections. No spirochætes were found in the inoculated guinea-pigs, and attempts to infect guinea-pigs with the cultures were similarly unsuccessful.

A number of Dutch workers have put forward some results which may in part confirm those of Whittingham and Couvy. At Deli, Sumatra, a febrile disease is endemic, it is characterised by slight yellowing of the conjunctivæ, albuminuria, and, less frequently, by splenic enlargement and rash. In some cases the general syndrome is that of dengue, whilst in others the disease rather resembles mild Weil's disease, which, in its typical form, is also met with in the same locality. Van de Velde, Konwenaar, Vervoort, and Baermann all describe the presence of leptospira in the blood as well as in blood cultures from these patients. The organisms were sometimes found to be pathogenic for guinea-pigs and sometimes not, and amongst the animals which succumbed certain showed lesions akin to those set up by *L. icterohæmorrhagæ*, whilst others were free from them.

Many of these cases would seem to form a link between typical Weil's disease, the non-icteric form of the malady and, possibly, dengue.

A similar, non-fatal, condition has been investigated by Fletcher,

in Malaya, who obtained positive direct blood-cultures in a high proportion of his cases. Many of these organisms were serologically indistinguishable from the organism isolated by Vervoort in the non-icteric cases just mentioned (*L. pyrogenes*). Fletcher encountered cases of true Weil's disease at the same time as the other leptospiral infections. He does not look upon the non-icteric form of leptospirosis as being identical with dengue, and makes of the presence of albuminuria in the former condition a distinguishing feature.

The suggestion has been put forward that the spirochætes seen in dengue and the allied fevers were artefacts, but though this explanation might apply to results obtained by the mere examination of films, or the dark-ground examination of cultures containing autolysed blood cells, it is difficult to understand how any experienced observer could be misled in cultural experiments, albeit most of the media used contained blood corpuscles which, in their disintegration, have repeatedly provided a trap to the feet of the unwary. The work of Harris and Duval upon American dengue (1924) does not clarify the situation, for although they obtained a regular pyrexial response in guinea-pigs, which was preceded by an incubation period of from two to five days, persisted for three to four days and was frequently succeeded, after its defervescence, by a short recurrence—a result which would accord fairly well with the experiments of Whittingham—they state that as a result of cultures made in Noguchi's medium they have obtained only "globoid bodies." These, on reinoculation into the guinea-pig, reproduced the same pyrexial reaction

A form of spirochætal infection, whose position is uncertain, occurred as an epidemic during the course of the late war at the French naval base of Lorient, in Brittany. The disease was at first described as icterohæmorrhagic spirochætosis by Manne, Cristau and Plazy, who, nevertheless, noted a great diversity in the clinical findings, and the presence of jaundice in only three out of thirty-one cases. Hæmorrhages, purpura, hæmoptysis and blood in the stools and urine, were amongst the symptoms seen, and enlargement of the liver was constant. The only feature

common to all the cases was the presence of large numbers of spirochætes, of the type of *L. icterohæmorrhagiæ*, in the urine. The organisms were seen early in the disease and were generally present at the time of the patients' admission to hospital. The mode of infection was obscure, but the infectious nature of the condition was evident.

The disease was investigated by A. Pettit, of the Institut Pasteur, who confirmed the findings of Manine, Cristaw and Plazy, but came to the conclusion that the disease was not one with the spirochætal jaundice at that time prevalent in the trenches on both French and British fronts. The points of difference were the ease with which the Lorient spirochæte could be demonstrated in the urine, and its presence at all stages of the disease including the early ones; the shorter and less regular appearance of the spirochæte when contrasted with that of spirochætal jaundice; the lack of protective antibodies in the serum of the convalescents against the *L. icterohæmorrhagiæ*; the clinical differences previously outlined—amongst 100 cases of this infection only five deaths occurred—and, lastly, the complete failure of the urine to cause infection of guinea-pigs. Pettit also attempted the infection of other animals with the spirochæte, but was unsuccessful.

It may be noted that the only inoculum used in these experiments, as far as can be gathered, was the deposit from the urine. This was frequently very rich in spirochætes, but, as is well known, the infectivity of the urinary spirochætes in Weil's disease is often slight. This, however, is not a serious criticism of the results obtained since a large number of animals were experimented upon, and some positive results should have been obtained if the animals were at all susceptible to the organism. The spirochæte was described by Cristau as having been seen in four cases in the cerebrospinal fluid, once in sanguinolent sputum, and once in smears from the liver obtained at post-mortem, but it was never detected in the circulating blood. The direct inoculation of animals with blood does not seem to have been undertaken.

The infection was localised to Lorient, with the possible exception of a few isolated cases of similar type reported in Paris. Its nature remains open to doubt, although it is assumed by some

writers to have been Weil's disease. The striking differences outlined by Pettit do not leave much ground for this assumption. The disease appeared in the middle of the war and seems to have disappeared again, its true nature never having been cleared up, nor does Pettit give any conclusions upon the matter, although figuring the organism in his book under the title *S. Pettiti*. The only actual connection of the disease with spirochætes was the demonstration of these in the urine, and possibly in a few very exceptional cases in the body. It is recognised that organisms of this type are occasionally seen in the urethra, and may appear in the urine, but it does not seem possible that the constancy and numbers of spirochætes found in the Lorient epidemic can be explained away upon these grounds.

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## DISCUSSION UPON LEPTOSPIRAL DISEASES

The work done upon these has been crowded into a very short space of time and there has been little opportunity for an interchange of experience or material between different workers. Consequently each investigation appears to be somewhat isolated and lacks the mellowing influence of first-hand criticism and suggestion from others engaged in the same field. Noguchi was the first who undertook a systematic comparison of the different strains of spirochætes isolated, but many others have been added to the list of pathogenic species since this was done. Fletcher (1928) has made a more up-to-date comparison. Upon serological grounds he classifies the named leptospira as follows.

Group 1 —*L. icterohæmorrhagæ* (Inada), *L. icteroides* (Noguchi)

Group 2 —*L. hebdomadis*, Type A

Group 3.—*L. hebdomadis*, Type B.

Group 4.—*L. pyrogenes* (Vervoort)

The leptospira of Couvy and Whittingham may belong to the fourth group.

Many of these diseases show a number of closely common features. All are caused by organisms alike in type, all are demonstrable by the greater pathogenicity of the organisms for the guinea-pig than for the human host, in all of them the infection appears early in the blood, from which it is absent when the disease is fully developed, and is found latterly in the urine, and in all, or nearly all, a rodent host has appeared to act as a reservoir of infection. To these features the *L. icteroides* in some respects and the leptospira described in dengue in others would appear to offer certain exceptions.

It is extremely difficult, from the reading of reported results, to form an accurate comparative view of the various conditions dealt with, but it would appear highly probable that many of them are related closely, *e.g.*, as typhoid and paratyphoid are

related, even if this be no closer. We may instance the relationship of "seven-day fever" to the "autumn fever" described in Japan by Koshina and his associates, and we may recall in this connection that Noguchi (1918), in studying the active immunisation of the guinea-pig by different strains of leptospira, found that in the finer degrees of this there was definite evidence of distinct immunological sub-types.

Upon the work of Noguchi in yellow fever judgment is still suspended. At the time of its publication, eight years ago, opinion set strongly in its favour, but in the interval which has elapsed, in spite of the confirmatory results reported by Noguchi and certain South American workers, there has been a definite reaction from this point of view, in the first instance mainly through the inability of other bacteriologists to find any essential distinguishing features between *L. icterohæmorrhagiæ* and *L. icteroides*. This opinion of the identity of these organisms has been subscribed to by Battistini (1925), Schuffner and Mochtar (1927), Puntoni (1927), Sellards (1927), and Brown and Davis (1927), amongst others who have investigated the matter by serological and immunological methods.

On the other hand, the very considerable mass of evidence brought forward by Noguchi and his co-workers cannot be lightly dismissed. If an error has crept in it is not altogether clear where the flaw lies, since the actual findings can hardly be rejected. The possible explanations are, either that Noguchi was dealing with Weil's disease, which was present though unrecognised in the epidemics in which his findings were obtained, or that Weil's disease and yellow fever are practically indistinguishable maladies.

With regard to the first speculation, it is to be remembered that Noguchi was throughout working in close contact with clinicians, to whom yellow fever was a familiar disease, and who should therefore be well able to distinguish it. At the same time, as he himself is careful to observe, the clinical differentiation between this disease and infective jaundice is by no means easy, especially in the less typical cases, and he expresses a doubt as to the practicability of making a positive differential diagnosis between the two in the

absence of bacteriological findings. It is with regard to these latter that the greatest difficulty occurs, for Noguchi has definitely considered the question of the identity of his leptospira with *L. icterohæmorrhagiæ* and deliberately concluded, upon all the available grounds, that the two are distinct, although he freely admits a close relationship. It is also to be noted that, in searching for the natural habitat of the organism, Noguchi examined numerous rats in Guayaquil and found leptospira in 67 per cent. of these animals. He definitely decided that he was here dealing with *L. icterohæmorrhagiæ* and not *L. icteroides*, upon fairly sharp protection experiments. Noguchi also concluded that the *L. icteroides* not merely survived but underwent pullulation in the body of the *Stegomyia fasciata*. Recently Gay and Sellards (1927) have put this matter to a more critical test by comparing the fate and length of survival, both of this organism and of the *L. icterohæmorrhagiæ*, in the mosquito's body. They carried out their experiments at 28° C., feeding the mosquitos upon guinea-pigs which had been infected with one or other of these leptospira. Their results are sharply contradictory to the inferential ones of Noguchi, since they find that no multiplication of the organism takes place, but that they begin to decrease immediately after having been taken into the insect's body and have died out in about a month. It is generally agreed that in the case of mosquitoes infected with yellow fever the infection probably persists for the rest of the insect's life. In the experiments of Gay and Sellards both types of leptospira experienced the same fate and died out at the same rate, so that in this respect also they would appear to be identical. No evidence whatever was forthcoming of the multiplication of the organisms which Noguchi postulated as a necessity for the stegomyia's infectivity. A similar result was obtained by Sawyer and Bauer (1928), both by the direct examination of the infected mosquitoes for the presence of leptospira and by cultural methods. They failed to demonstrate a survival of the *L. icteroides* for more than nine hours; all efforts at recovering the organism after a longer period failing. Any breakdown of the chain of evidence of the ætiology of this disease in respect of the mosquito would be a fatal flaw in Noguchi's argument, since the

*role* of this insect is one of the most firmly established facts in our knowledge of yellow fever.

On the other hand, it is possible that both of these diseases are due to similar organisms, whose distinction is slight and perhaps more easily made in the freshly isolated organism than in old laboratory strains. It is generally admitted that the virulence of leptospira is a very variable quantity, and one school of bacteriologists holds that the pathogenic leptospira are developed from the saprophitic *L. biflexa* through the chance acquisition of a parasitic existence. The experiments of Brown and Davis show a complete serological distinction between these organisms and the pathogenic leptospira, so that if such a transformation into *L. icterohæmorrhagæ* is an actual process, a change in serological characters must accompany it. This, of course, may be so and, as certain evidence suggests, variation in virulence and associated serological characters may be less fixed in this species than in most of the common bacteria. It is becoming more and more evident that spirochætoses (leptospiroses) are of commoner occurrence in tropical countries than has been generally recognised, and are due to organisms of several serological types, whilst, as recent work shows, their clinical manifestations may be protean. It is, of course, possible that yellow fever may belong to this group, and approximate more closely to Weil's disease than to the less severe simple febrile disorders. The great objection to such a view in the case of yellow fever, even when all allowances are made for possible variations between closely allied species of leptospira, is the lack of confirmation of the existence of the leptospira in the stegomyia, and in the second place, the recently reported work of Stokes, Bauer and Hudson, upon West African yellow fever, in which long and systematic investigation failed to show the presence of either spirochætæ or of any transmissible infection in the gumca-pigs which were injected with patients' blood. All the cultural and animal experiments carried out by these investigators were entirely negative, with the exception of attempts to infect *Macacus rhesus* monkeys, which succeeded. Both puppies and chimpanzees were found to be resistant to the virus. In the infected monkeys, although definite disease developed, leptospira could not be

demonstrated. Such a result is entirely opposed to our second hypothesis, which is now somewhat threadbare. It is evident that an entire and independent re-examination of the whole matter in an area such as West Africa, far removed from the one in which Noguchi's observations were made, is an essential step in the further study of this problem. That step is now in process of being taken.

Turning from the question of yellow fever, there remain the other leptospiral febrile infections in which a good deal of confusion still exists. The work of Whittingham and Couvy upon sand-fly fever or dengue, although not yet established, will, if it is confirmed, help the extension of our ideas upon these spirochætal diseases. It would result in the establishment of a group of such maladies in which the contagion is transmitted by insects and not, as far as is known, dependent on a rodent host for its persistence or propagation. It may be recalled that Couvy's cases of dengue developed severer clinical manifestations towards the end of the epidemic than were seen in the earlier stages, and that some of them had jaundice and one *vomito negro*. Whittingham, in discussing the clinical symptomatology of the Maltese cases of pappataci fever, says that its manifestations vary at different periods from a mild influenza-like disease to severe forms which are termed "yellow fever". Assuming that these differences really represent variations in one and the same disease, the observations may be of some significance in pointing to a general variability in the clinical picture in leptospiral maladies. The results of the Dutch workers in Sumatra are also worthy of consideration in this connection. Here a graded series of cases has been reported, varying from a simple febrile disorder up to severe infections of the type of Weil's disease; the pathogenicity of the spirochæte, however, fell short of that of *L. icterohæmorrhagica*. In the case of undisputed Weil's disease, the clinical manifestations and mortality are recognised as being very variable. The death rate in Japan is between 30 and 50 per cent.; in France, during the war, it was not more than 5 per cent.; whilst Buchanan, in a relatively small series in Edinburgh, found it to be as high as 25 per cent.

Similarly the proportion of non-icteric cases varies considerably in different outbreaks.

In consonance with such clinical findings, the labile character of the virulence of most of the investigated forms of leptospira has been remarked upon repeatedly, the property is readily lost in culture, and has been restored by passage. Noguchi has found that in the continued propagation of *L. icteroides* by animal passage, the clinical and morbid anatomical features of the disease changed; it assumed a more hæmorrhagic character, jaundice became less prominent, and finally death was produced by acute septicæmia.

Upon the matter we have been considering, we may conclude that substantial claims have been made out for the existence of a number of leptospiral infections in different parts of the world, whose clinical boundaries are somewhat ill-defined, and whose manifestations differ in different areas. The exact relationships of the causal organisms are not as yet well established, but would seem to be close.

#### A NOTE UPON THE THROMBOCYTOBARIN (ADHESION) PHENOMENA

In 1917 Rieckenberg noticed that in the citrated plasma of rats which had recovered, or been cured, from an infection with *Trypanosoma brucei*, adhesion of platelets to the surface of the trypanosomes occurred when these were present together. He further found that this was a specific phenomenon, and that, whilst it occurred with plasma, it was not produced in the serum separating from clotted blood. Kritchewsky and Tscherikower (1925) further investigated the phenomenon and found that the specificity depended upon the fluids of the blood and that the platelets were the mere mechanical indicators of the effect. It was also found that the reaction was an ordinary antigen-antibody one, which might be produced in an animal's humours by the injection of dead trypanosomes or spirilla, whilst Krantz (1926) showed that bacteria would serve as indicators equally as well as platelets.

Davis and Brown (1927) investigated this test, and found that



A.



B

FIG 21.—The adhesion phenomenon with leptospira. Negative (A), Positive (B) (Davis and Brown.)

it applied both to trypanosomes and to spirochætes and, further, that living particles were not necessary for the demonstration of

such adhesion, but could be replaced by inorganic particles ; in some of their experiments they used a suspension of gamboge. The immune-body was highly thermostable, and its production in rabbits and guinea-pigs was demonstrated with ease. As had been found by previous observers, old serum is not effective in the reaction, but a trace of complement, which may be quite small, suffices for its activation. These workers carried out the test by mixing, in a small test tube, a small measured quantity of fresh antiserum and an equal quantity of a suspension of the spirochæte or trypanosome, in which the organisms should be discrete and free from clumps. A thin suspension of bacteria in saline is added as an indicator, and the mixture incubated at 30° C. for about twenty minutes. A drop is then examined by dark-ground illumination. A positive reaction is evidenced by the crowding of the bacteria on to the surface of the protozoon, and a negative reaction by the absence of any such adhesion (Fig. 21)

In the case of old stock sera reactivation may be obtained by the addition of traces of complement. It is, of course, in all cases necessary to exclude the possibility of natural antibodies, which in the case of leptospira are found in many of the lower animals. It is also necessary that no marked lytic effect shall be produced by the serum in use. Brown and Davis noted that their sera did not give the reaction if much diluted, and in most cases used undiluted serum.

By means of the adhesion test they were able to obtain sharply-marked serological differentiation between the saprophilic *L. biflexa*, and *L. hebdomadis* and *L. icterohæmorrhagica*. With regard to *L. icteroides* they found it to react in the same way as *L. icterohæmorrhagica*.

The phenomenon has been variously referred to as the "thrombocytobarrin reaction" or "Rieckenberg reaction." Davis and Brown propose for it the term "adhesion phenomenon," and suggest that it may be of practical use in the serological study of trypanosome and spirochætal infections, since in the differentiation of leptospira the agglutination reaction, except in the experience of Pettit, is not very satisfactory. They point out that Levaditi and Mutermilch (1919) observed an analogous effect

between leucocytes and trypanosomes in immune animals, and noted that it was a physico-chemical phenomenon independent of the living state of the leucocytes. From the published accounts, this phenomenon, which appears to be of some promise in the serological diagnosis of protozoal infections and has been applied by Messik (1927) to *Leishmania tropica* infections, would seem closely allied to the other immunity reactions involving a surface alteration in the antigen.

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## NEUROTROPIC STRAINS OF *T. PALLIDUM* AND THE *T. CUNICULI*

The vagaries of the spirochæte of syphilis, with respect to its action on the nervous system, has in the past not infrequently led to the acceptance of a suggestion that specially neurotropic strains of the organism may exist. These, it is supposed, tend not towards tertiary lesions, gummata and the like, but towards the more degenerative, parenchymatous, types of nervous disease, paralytic dementia and locomotor ataxia. Babinski seems to have been the first to originate this idea, which has obtained a good deal of acceptance amongst clinicians as a result of the rather remarkable divorce which may be observed between tertiary lesions and parasymphilis. The French school have found the view attractive, and Mott, in this country, supported it. Amongst clinical evidence, the following case, quoted by Morrell-Lavallée, has become almost classical, as suggesting a constant neurotropic tendency in a single strain of the infection

### *Marthe "X"*

May, 1870   Mis- tress of "A" (medical stu- dent) Gave him syphilis. He died in 1873 of syphilitic men- ingitis	December, 1871   Mistress of "B" (medical stu- dent) She gave him syphilis. He died in 1888 of general para- lysis	January, 1872   Lived four years with "C" (medi- cal student) He died in 1882 of general para- lysis	Later   Mistress of "D" (chem- ist) He died in 1800 of general paralysis	Still later   Mis- tress of "E" (engineer) He died of "folle syphilitique"
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The matter, which upon clinical grounds seems well founded though open to other explanations, was taken up experimentally by Levaditi and Marie. They claimed that the strains of spirochætes present in cases of general paralysis, upon inoculation into rabbits caused only mild superficial cutaneous lesions, whilst those obtained from primary chancres, or in the secondary manifestations, gave rise to the better-known and more severe types of rabbit lesion.

The view developed that not only could marked differences in virulence exist between such strains of the syphilitic spirochæte, but that these might also be reflected in corresponding immunological differences. For instance, Fournier and Schwartz (1928) recounted an example in which two strains of spirochætes (both isolated from chancres) gave markedly different results upon inoculation into rabbits. The one set up superficial, impetiginous, and crusted lesions, whilst the other gave rise to the large, indolent, ulcerous lesions typical of rabbit syphilis. They further found that recovery from infection with the one virus conferred no immunity against the other. This was a weighty matter; but the value of such observations has been called in question by the discovery of a spontaneously occurring venereal infection in rabbits, associated with a spirochæte morphologically indistinguishable from that of syphilis.

The condition was first discerned by Ross (1912), who observed a disease in rabbits characterised by ulcerous lesions upon the genitals and mucous membranes and suggested that a spirochæte might be responsible. Bayon (1918) found this to be the case, and identified an organism which could not be distinguished from *T. pallidum*. Jacobsthal (1920), amongst others in Germany, studied this disease and named the organism *Spirochæta paralus cuniculi*. Although clinical and immunological differences between this organism and *T. pallidum* were noted, these were not altogether sufficient to make a reliable differentiation between the two. Noguchi (1922) found the disease occurring spontaneously in American rabbits, and made a careful study of the infection. He found the typical lesions to be papulo-squamous, ulcerative ones, generally affecting the perineal region and histologically not unlike those of experimental syphilis. The disease was capable of transmission from rabbit to rabbit by coitus, but the deep inoculation of infectious material into the scrotum did not give rise to the typical ulcerated and indurated syphilitic lesion. In rabbits experimentally infected with syphilis the Wassermann reaction was positive, but in no case did infection with *T. cuniculi* produce this result.

Amongst recent workers Warthin, Buffington and Wanstrom

(1928) have studied the venereal spirochætosis of American rabbits, and Adams, Cappell and McCluskie (1928) that of British rabbits. Both sets of observers have confirmed the earlier findings and demonstrated the essentially superficial nature of the lesions produced by *T. cuniculi*. The disease is curable by local mercurial inunction, or by the intravenous injection of salvarsan.

The existence of such a spontaneous disease, and its close similarity to syphilis, was immediately put forward as the clue to the mystery of the immunologically differing strains of the treponema found in the rabbit, and it was freely suggested that the neurotropic strains described by Levaditi and Marie, which were found in lesions produced in these animals by inoculation with brain material from general paralytics, were none other than the *T. cuniculi* which had accidentally complicated their experiments, and of whose existence these workers were at the time ignorant. Levaditi and Marie (1919) returned to the subject and demonstrated again the differences in virulence between their "neurotrope" and "dermatrope" (chancre) strains of treponemata, and also the absence of any reciprocal immunising effect between them. Taking up the question of the *T. cuniculi* they found no crossed immunity between this organism and the "dermatrope" strain of *S. pallida*, but upon repeating the experiments with the "neurotrope" organism did not succeed in absolutely establishing a like independence, although they make out a partial claim to have done so. Levaditi and Marie reiterate their conviction that parasyphilis is a separate and distinct type of disease, due to strains of treponema differing biologically and morphologically from those found in the common cutaneous and visceral manifestations of syphilis. In the view of others, however, and in consideration of the difficulty which must be encountered in infecting rabbits with material from parasyphilis, on account of the sparsity of the organisms and the difficulties in obtaining infective material, the more probable explanation of their experimental results is that the avirulent "neurotrope" strains of spirochæte were none other than the *T. cuniculi* of natural rabbit pseudolues.

This view is supported by experiments recorded by Danila and Stroe (1923), who succeeded in isolating a strain of *T. pallidum*

from the cerebrospinal fluid of a case of juvenile general paralysis. This organism, though of authentic neural origin, displayed no more respect towards the rabbit than did spirochætes derived from chancres. In other words, it was just as "dermatrope" as the syphilitic organisms to which Levaditi applied this term. With regard to the *T. cuniculi* these authors confirm the observations of Levaditi and Marie that the organism is non-pathogenic for man, thus differing from the *T. pallidum*. They also observed a complete absence of any crossed immunity between it and the syphilitic organism.

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## CHAPTER XV

### LOCAL IMMUNITY AND THE WORK OF BESREDKA

THE strife and arguments which circled around the theories of immunity twenty years ago are largely forgotten to-day. The fierce differences between the cellular school, of which Metchnikoff was the great protagonist, and the humoralists, whose home was largely in Germany, do not vex the mind of the modern student who lives in the tranquil atmosphere resulting from an agreed peace—with honour. These matters may be read by the curious in Metchnikoff's great work upon "Immunity in the Infective Diseases." Even though the partisans of a purely phagocytic theory, and a purely humoral one, have found common ground and have to-day composed their differences, knowledge of the mechanism of bodily defence is still very inexact. One may almost regret the shelving of the old controversies since, with the passing of their stimulating influence, interest in the processes of immunity has waned. Research in immunity, be it also said, has opened up so many by-paths of importance, and produced so many useful by-products in the shape of clinical tests, that for a period the main issues were clouded in the mass of complexities involved by the elaborate technique of these processes. It is the merit of more recent workers in this field that they are again turning their attention to the fundamental problems of the relationship of the living body to the invading parasite.

Amongst other of these workers, Besredka must be given a notable place both on account of the interest which his views arouse and on account of the practical applications which have been drawn from them. Briefly put, his main thesis is that the defensive mechanism of the body, in regard to the initial entry of pathogenic organisms, is less an affair of general resistance common to all tissues than a local matter, concentrated in the tissue which

is primarily the seat of disease and through which the organism normally gains entry into the body. The view is very largely based upon experiments with anthrax, in which disease Besredka holds that for infection to occur a primary invasion of the skin is essential. This is the path by which the infection normally occurs, and if immunity be established this immunity is a local process residing in the skin. It is therefore a local tissue-immunity, and does not rest upon circulating phagocytes or antibodies in the usual sense at all.

These views Besredka bases upon the following considerations. The difficulty of immunising small laboratory animals to anthrax, guinea-pigs especially, is well known. If anthrax bacilli, even not fully virulent (*e.g.*, Pasteur's *deuxième vaccin*), be applied to the shaven and excoriated skin of a guinea-pig the animal dies of anthrax. But, according to Besredka, if the *premier vaccin* be applied to such a shaven area the guinea-pig survives, a slight and transient local inflammation being the only visible result. If now the *deuxième vaccin* be applied to the raw skin of the same animal, a local self-limiting reaction alone occurs and the animal recovers, whereas a control animal succumbs in the ordinary way to a generalised infection. The animal which has recovered from treatment with the second vaccine is now highly immunised, and will resist not only a similar skin inoculation with fully virulent cultures of anthrax but also a subcutaneous injection of a small quantity of such a culture. The solid immunity which has been set up by these procedures may be imitated equally well by injecting the vaccines intradermally, instead of applying them to the raw surface. Such a degree of immunity, as these procedures result in, Besredka declares to be unattainable by the ordinary methods of graduated subcutaneous doses.

Besredka holds the explanation of these results to be that the animal's initial sensitivity to the infection lies in its cutaneous tissues. Where such an animal dies, following upon a subcutaneous, intramuscular, or intraperitoneal injection of anthrax bacilli, he believes that this is due to the inevitable soiling of the skin puncture with the organisms and that the fatal infection starts from this locus. He supports such a view by adducing the

results of experiments in which the organisms have been introduced into trachea and peritoneal cavity in enormous doses, without the skin being in any way involved, and the animals have survived. At the same time such animals show no immunity to subsequent cutaneous test-inoculations. The skin, for him, is the vulnerable organ ; the others can look after themselves. Immunise the skin, and you have immunised the whole animal, by blocking the path of entry of the bacteria into the only receptive tissue. This is Besredka's thesis.

In consonance with this general notion of localised tissue-immunity Besredka finds that the ordinary antibodies are absent in animals thus immunised and that their serum is incapable of conveying passive immunity to other animals. This is not an undisputed matter, but it would not appear a very vital one to the general argument if it is agreed that the antibodies, if present, are only to be found in small amounts and in lesser concentrations than are to be met with in animals vaccinated by intravenous or subcutaneous methods and less effectively immunised. The complex mechanism of immunity, in its artificial phases, cannot be expected to be limited to one of its possible manifestations to the extent which Besredka claims, although in a given case any single one of these may predominate.

The reason for the successful accomplishment of the immunisation of sheep by the subcutaneous injection of the organisms, according to the method of Pasteur, and the failure of this method when applied to other animals, is not easy to understand in the light of the views here set forth. Besredka states that the successes are due, not to the subcutaneous inoculation, but to the accidental and unpremeditated inoculation of the skin which occurs when this is carried out. He believes that the bacteria track back along the path of entry of the needle, and affect the skin cells from which the immunity is derived. This explanation is anything but satisfactory, since it would appear to apply mainly to one species of animal.

It is objected that in many of the cases in which laboratory animals are inoculated with anthrax the disease pursues an acute course, and is septicæmic from the beginning, there is in such

cases no evidence of the cutaneous involvement which the theory we are discussing implies. Besredka answers that such a result is only observed in the case of cultures of exalted virulence, encapsulated bacilli, or bacilli acting in the presence of aggressins. He claims that these fatal effects are due largely to the presence of toxins, although admitting that the *in vitro* production of anthrax toxins has not been effected. In a discussion upon these views at a meeting of the Royal Society of Tropical Medicine and Hygiene, in 1924, Ledingham suggested that the relatively high immunity of certain tissues towards anthrax bacilli, which has been demonstrated by Besredka and forms the basis of his thesis, is less a specific effect than due to the greater development of local defensive mechanisms, notoriously effective in the peritoneum and of a low order in the skin. Views of a similar nature have also been put forward by Gratia. To this it is replied that the phenomenon is not of general applicability for all bacterial species, as it should be if this were the correct explanation; the cholera vibrio, for example, is more highly pathogenic by intraperitoneal inoculation than by subcutaneous injection.

The experiments of others, who have followed Besredka, show a measure of confirmation of his work upon anthrax. Balteano (1922), in the case of rabbits, found a lesser susceptibility in the blood, pleura, peritoneum, and subcutaneous tissues than was exhibited by the skin, and he also successfully reproduced Besredka's experiments upon the immunisation of the guinea-pig by the application of cultures of attenuated virulence to the shaven and abraded skin. In the case of larger animals Vallée (1923) found that the ox, which is relatively insusceptible to experimental anthrax, could be infected more easily by intradermal inoculation than by the subcutaneous method, a result which is in keeping with the general ideas set out above. Plotz (1924), in the case of the rabbit, confirmed Besredka's views upon the lack of sensitivity of tissues other than the skin by introducing capsules, sealed capillary tubes, etc., beneath the skin and rupturing them when healing had taken place. The animals survived large doses of strains of anthrax bacilli which were found in certain instances to be fully virulent upon cutaneous inoculation. These results,

however, were not fully controlled. Other observers have not obtained the same results in like experiments. It is claimed by Besredka that these failures are to be explained by the incomplete healing of the wound which is necessary for the introduction of the organisms, which when the capsules are broken allows access of the bacteria to the vulnerable cutaneous tissues.

The experiments of Balteano and Plotz, with glass tubes and capsules introduced into the subcutaneous tissues, were repeated with great care by Panton and Benians (1925). They fully confirmed the negative effects resulting from the liberation of the buried bacteria, but their work disclosed numerous fallacies bearing very acutely upon this. In the first case they found that in many instances the anthrax bacilli had become attenuated and avirulent, whilst they also point out that some degree of encapsulation of the container had occurred. It was also difficult to make sure that the bacteria were all liberated when the tubes and capsules were fractured. Under such circumstances the results are deprived of most of their value, since not only must they be discounted by reason of the attenuation of the bacteria and the uncertainty of the dose, but it must also be recognised that such bacteria as are liberated are brought into contact, not with normal subcutaneous tissues, but with a reactive granulation tissue, which is a notoriously capable resister of infection. As a result of other experiments Panton and Benians concluded nevertheless that Besredka's claim is to some extent just. They found that in certain instances the rabbit could tolerate a dose of anthrax bacilli beneath the skin which proved fatal on cutaneous inoculation, and they therefore agree that the receptivity of the skin is greater than that of the subcutaneous tissue. In opposition, however, to Besredka, and in agreement with Gratia, they found that subcutaneous inoculation, effected without any soiling of the skin, was productive of a definite degree of immunity.

Results of a similar nature, upon local streptococcus immunity, have been obtained by Gay (1928), who found that an animal which could be rendered resistant to a cutaneous inoculation of these organisms was not protected against inoculation by another route, and that a similar local protection could be demonstrated

by intrapleural inoculation. He concluded that a specific local immunity could exist as well as a general immunity.

As a result of Besredka's experimental work, his methods have been introduced upon a considerable scale into veterinary practice in France, and in countries in which the influence of French medicine is predominant. In the case of the horse cutaneous vaccination against anthrax has been extensively practised in the French forces in North Africa, and Nicolas (1925) has reported that it is safe in practice, safer in fact than the Pasteurian method, which involves the loss of a certain number of animals in the course of immunisation, and that the results of its use in areas where anthrax is prevalent have been excellent. Newodoff has used the same method in cattle, and Velv (1927) has given it an extended trial in the case of sheep in Morocco, and finds many advantages in the method. Amongst these he notes that a satisfactory immunity can be obtained by a single injection of about a fifth of the dose required for immunisation by the subcutaneous route, and that this immunity develops rapidly and is at least as complete and durable as that obtainable by any other method.

### **THE INTESTINAL TRACT**

In the case of anthrax the question may well be put that since in the spontaneously occurring disease of animals infection is by way of the intestine, what is the significance of the theories of Besredka which bear so largely upon the susceptibility and resistance of the skin? The classical experiments of Pasteur, Chamberland and Roux demonstrated that for the invasion of the body by the alimentary route some breach of surface in the mucosa was necessary, which they envisaged as occurring through the abrading action of rough and sharp particles, such as the beards of grain, etc. Besredka believes that the intestinal mucosa in this disease behaves like the skin, and is itself an especially vulnerable tissue in respect of the anthrax bacillus and capable of acquiring a local immunity with it.

The views of this investigator which we have considered up to now, were in reality developed from earlier experiments

which suggested that immunity to infections which primarily involve the intestinal mucosa might be obtained by local vaccination of this tissue by means of the oral administration of vaccines. From this point of view they were gradually enlarged to cover other infections which are essentially localised to special tissues. We may for a moment follow the development of these ideas

Besredka, in the course of some investigations into the vaccination of mice against *B. paratyphosus B*, attempted to infect these animals by the buccal route, but failed to do so. One day, being short of control animals in the testing of the immunity produced by injected vaccines, he made use of animals upon which this abortive experiment had been made a month previously. Inoculated with a certain lethal dose of *B. paratyphosus B*, the controls survived! Taking up the thread of this unexpected result, Besredka determined that animals which had ingested the bacteria developed immunity after a latent period of about ten days. This was most effective in cases in which living virulent organisms had been absorbed, it was less marked where sensitised bacteria were given, and least well developed after the ingestion of dead bacilli.

It is well known that the picture of typhoidal disease cannot be produced with any fidelity in rabbits, although these animals are readily killed by injected bacilli of this group. Besredka attempted to weaken the intestinal barrier by the administration of ox bile and states that when this is done alimentary infection with *B. paratyphosus B* succeeds. A fatal paratyphoid infection ensues, and at post-mortem the small intestine is filled with greenish, fluid contents; the epithelium is desquamated; the Peyer's patches swollen, the gall-bladder distended, and the bile infected with paratyphoid bacilli. It may be noted that the measures taken to produce this result are very drastic. The animal is given 10 c.c. of a mixture of bile and powdered liquorice at 5 p.m. and the following morning, at 10 a.m., the animal having been kept fasting, a second similar dose of 10 c.c. of bile. Two hours later the bacteria are administered. It has been shown, both by this author and by others, that the effect of such dosage with bile is to greatly increase the permeability of the intestine and to lead to the absorption of substances in an unaltered state which would not otherwise pass

through the epithelium. Dietrich was able to show this in the case of tetanus toxin.

It had previously been shown by Loeffler, in 1906, that protection against *B. typhi murium* could be conferred upon mice by feeding them with dead cultures of these organisms; and Wolf (1908) obtained a similar result by the use of a live but avirulent strain. Bruckner (1910) also immunised mice against injected paratyphoid bacilli by feeding them upon living bacteria of the same species. In attempting to apply these results to the vaccination of man, Besredka was faced with the difficulty that such immunisation as results from the ingestion of dead bacteria is not very effective, and on the other hand the use of living organisms was obviously impossible. He consequently turned to his experiments upon the administration of bile along with bacteria, in the hope that by such a manoeuvre it might be possible to modify the permeability of the intestinal wall so as to allow the absorption of dead bacteria and thereby to effect an adequate immunisation. By applying the same technique as had been used for the production of a paratyphoid infection, but by substituting dead organisms for the living ones, Besredka found that the rabbit could acquire immunity to intravenously injected paratyphoid bacilli; and he believes that such a result is attributable to the damaging effect of the bile upon the intestinal walls. He further found that this immunity was rapidly developed, being effective in the short space of three days, and that it was not associated with any notable production of titratable antibodies.

As a result of these experimental findings he concludes that the immunity produced in this way is not a general immunity, in the sense in which this is usually understood, *i.e.*, dependent upon circulating antibodies, but is a local immunity residing in the intestinal cells, which in such an infection play the rôle of an initial susceptible tissue, in the way in which he conceives the skin to act in anthrax. This cannot be regarded as proven. The production of antibodies is a matter which most of the earlier workers upon the effects of intestinal administration of antigens concentrated a good deal of attention, and they were usually able to detect them in cases in which immunity was definitely

established (Courmont and Rochaix, Kutscher and Meinicke; Bruckner, etc.).

Webster (1922) has to some extent tested Besredka's views in his experiments upon the immunisation of mice with *B. pestis caviæ* (mouse typhoid), given by the mouth. Whilst finding that the oral administration of both live and dead organisms is productive of immunity, Webster finds no evidence of this being a local one. The immunity produced was as effective against organisms injected intraperitoneally as against organisms administered *per os*, and, conversely, the immunity produced by the injection of organisms into any part of the body was equally effective against later infection by whatever route this might be attempted. Moreover, agglutinins were demonstrable in the serum of a number of orally immunised mice.

Although in the cases in which antibodies have been detected in the blood, as a result of the oral administration of organisms, these have frequently only been detected with difficulty, this cannot be taken to indicate an insufficiency of immunological response on the part of the animal, since our methods of antibody demonstration leave much to be desired. Furthermore, Besredka's doctrine of resistance depending upon the local immunisation of a special tissue, normally sensitive and the starting point of the infection, does not seem applicable to the experimental paratyphoid infection from which it was so largely built up. There is no evidence that the death of rabbits which results from intravenous inoculation with these paratyphoid bacilli is due to an infection commencing or especially localised in the intestine. The fact that the organisms are found after death in the gall-bladder, and upper intestinal contents, does not of necessity denote anything more than their elimination by the liver.

In dysentery, to which the same reasoning has been applied, the case is somewhat stronger. Here we have to deal with a disease which is pre-eminently a local affection, the organisms showing little ability to invade the tissues deeply. Although the disease cannot normally be reproduced in the smaller laboratory animals, which are usually refractory to the organisms when given by the mouth and succumb, when these

are injected intravenously, to the toxic and paralytic effects of the bacteria, Besredka states that with certain strains of *B. dysenteriae* (Shiga) of high virulence, dysenteric lesions result if the organisms are given by the mouth to fasting rabbits. In furtherance of his general thesis he bases a view that the bacilli have a special tissue affinity for the intestinal mucosa, chiefly upon the fact that after both subcutaneous and intravenous injection they appear in the intestinal contents. He does not seem to consider that their presence here—and he especially emphasises their presence high up in the small intestine and in the gall-bladder—may, as in the case of his typhoid experiments, merely indicate their elimination by the liver rather than a special and obscure “enterotropism.” A like condition has been encountered and especially studied by Sanarelli in the case of cholera infections in young rabbits (1920).

In the experimental application of these views Besredka found that the ingestion of dead dysentery bacilli, following the technique described in the case of his paratyphoid experiments, led to the development of immunity against intravenously inoculated bacilli. In most cases antibodies made their appearance. Previously Zetlin (1905) had shown that the oral administration of dead dysentery bacilli to rabbits led to the development of agglutinins, and suggested the possibility of applying the method to man, and Shiga (1908) had obtained similar results. More recently the matter has been put to the test by Kanaki (1921) in this country. He found that large doses of killed bacilli (*e.g.*, one-fifth of the growth from the surface of a Roux bottle) produced a certain immunity in rabbits against a test dose of *B. dysenteriae* (Shiga) given intravenously. This dose was just on the border-line of a lethal one, killing some, but not all, of the controls. The treated animals developed a low agglutinin titre in their serum. Smaller doses of bacilli did not afford protection, and the protection which was afforded was in any case much less certain than that given by the subcutaneous injection of a carbolised vaccine.

The application of these principles to man, if their successful application were possible, would effect an important step of especial value in dysentery. In the typhoidal diseases the

experiences of the late war leave little room for doubting the great efficacy of subcutaneous inoculation, but this method has never been put into successful practice in dysentery on account of the severe reactions which occur. Although various modifications have been suggested, by which it is claimed that the toxicity of the organisms is reduced or abolished, none of these has ever found general usage.

Besredka claims that an effective immunity against the typhoidal diseases can be produced in man by the ingestion of dead typhoid bacilli, administered subsequent to the taking of a small quantity of bile on an empty stomach. By comparison with the technique adopted for rabbits the dose of bile administered to man would appear absurdly small and of doubtful effect. This method has been given a trial in certain post-war outbreaks of typhoid, in France and Roumania, and good results are reported. With these we do not need to greatly concern ourselves, since the efficacy of the subcutaneous method is not in doubt, and the published results for the alternative method are not sufficiently extensive or striking to suggest abandoning it.

In the case of dysentery the matter requires more careful consideration. Calmette (1928), in a review of the whole subject, points out that Shiga had attempted the vaccination of the human subject against dysentery, by means of the oral route, in asylums in which the disease was endemic and in other localities in which it was prevalent. The results were said to be good. Nicolle and Conseil put the matter to the experimental test on a limited scale in 1922. After pointing out that the proof by statistics of such a method of immunisation was too slow a business, whilst that by the production of antibodies was of uncertain and debatable significance, they turned to the direct test of protection upon man as being the only satisfactory way of solving the problem. Their experiments, which were carried out in Tunis, were pursued with considerable courage and determination. The first difficulty, in the case of dysentery, was to arrange for satisfactory controls. To this end, and their results may be remembered in connection with Besredka's and Sanarelli's ideas upon "enterotropism," they injected live Shiga bacilli intravenously into volunteers and

produced a general toxic reaction only—not dysentery. They then turned to the oral administration of the bacteria, and found that whilst five out of five Europeans experimented upon contracted dysentery, six attempts upon natives were without any positive result, but by the rectal injection of bacilli they succeeded in infecting three out of six natives. A preliminary administration of croton oil did not favour the infection!

Nicolle and Conseil then attempted to prove Besredka's thesis upon the North African natives, by giving selected volunteers three successive doses of 0.05, 0.1 and 0.2 gram of dried, killed, Shiga bacilli, whilst fasting: the bacilli were given in cachets, as recommended by Besredka, and the treatment spread over four days. Fourteen or fifteen days later an attempt was made to set up an infection by way of the rectal and oral route, or both, with a live and virulent strain of the organism. The results of this heroic experiment were that out of nine vaccinated subjects none contracted the disease, whilst out of twelve unvaccinated controls three became infected. The most striking observations to be made upon this is the marked immunity possessed by the native which, as the authors remark, rendered the results of the experiment inconclusive.

Not to be foiled, Nicolle and Conseil repeated the test upon Europeans, whom their previous experiments had shown to be more susceptible to buccal infection. Their stock of volunteers was running low and it was only possible to find four: two experimental subjects and two controls. The subjects of the experiment, in a period of five days, ate four doses of 100 milliards of dead Shiga bacilli coming from a recent case of dysentery. The diet was taken after a fast of some twelve hours which was maintained for a further period of three hours after the bacterial meal. Fifteen and eighteen days later the test was completed by the ingestion of ten and twenty milliards of live organisms, the like doses being given to the controls. The vaccinated subjects remained healthy and one developed a feeble concentration of agglutinins in his blood: the controls both contracted bacillary dysentery. As a result of these limited experiments Nicolle and Conseil concluded strongly in favour of the Besredka method.

On the statistical side it may be recorded that, in a barrack epidemic at Versailles, Besredka vaccinated 546 soldiers, amongst whom 42 cases of dysentery subsequently occurred, *i.e.*, 7.6 per cent. 1,070 men in the same garrison were unvaccinated and produced 297 cases of the disease, *i.e.*, 27.7 per cent. Favourable results have also been reported from Russia and Greece. In one refugee camp 1,000 persons were vaccinated and showed 12 cases of dysentery, whilst in another 1,768 who were not vaccinated gave rise to 56 cases. In other instances which have been reported the figures are not so clear cut.

Whilst these theories and experiments are to some extent novel and attractive, the practical deductions which are being drawn from them must be viewed with caution. It does not appear open to doubt that some protection against intestinal disorders can be set up by the oral administration of bacteria; but it must be remembered that in the animal experiments, upon which so much of this work is based, these doses have required to be relatively enormous to be in any degree efficacious. What, then, the effect of much smaller doses is, in protecting man against the natural risks of infection, in times of epidemic or in endemic areas, yet remains to be proved. Such field trials of these methods as are at present available are not altogether conclusive, and the degree of strictness exercised in the control is uncertain. Under the circumstances it is therefore not possible at the present time to pronounce upon their usefulness.

### ANTIVIRUS

It is common knowledge that media upon which bacteria have grown become unsuitable for the propagation of other strains of the same organism. A broth which has been utilised for the growth of *B. coli* will not, when filtered free from these organisms, serve as a medium for a fresh culture of the same bacillus. An agar slope, upon which streptococci have been grown, will not serve as a medium for a second streptococcus after the colonies of the first have been washed away. This is a very old observation, and upon it Marmorek largely based his contention of the unity of the

streptococci. The reason for this phenomenon is not particularly clear. It might, of course, be said that it is simply due to an exhaustion of pabulum, but this explanation will hardly hold since the effect is a specific one, it is demonstrable even with young cultures, and bacteria other than those of the type first grown on the medium will flourish abundantly. Also the use of media in which certain organisms have been previously grown is a well-recognised manœuvre in bacteriological technique. Such specificity is difficult to link with the growth requirements of the organism, which, from most points of view, admit of very considerable variations in media.

Besredka believes that this inhibitory effect is due to a specific soluble substance, secreted by the organism, which prevents its development and also has the power of inhibiting its specific pathogenic effects *in vivo*. This substance he finds to be remarkably thermostable, resisting a temperature of 120° C. for twenty minutes. Besredka maintains that by the application of such a preparation to the shaven skin a local immunity against the specific organism immediately follows. These ideas have been put into practice in the case of septic infections, and sterile filtrates of cultures of some days' growth are used as wet dressings under the name of "antivirus" The material has been put on the market by certain commercial firms. The effect, if any, cannot be a bacteriophagic one, since the active principle is thermostable. There is no clear evidence of its value apart from pious opinion.

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## CHAPTER XVI

### RECENT WORK IN CONNECTION WITH DIPHTHERIA

THE epidermal reaction to injections of minute doses of diphtheria toxin which occurs in susceptible persons (Schick test) is so well known as to require no further description here. As is also well known, one of the chief practical outcomes of this method of discerning who are the susceptible and who are the immune in a given population has been the introduction of active immunisation by means of toxin-antitoxin mixtures. This procedure was first applied to the human subject, in the case of diphtheria, by von Behring (1918), who used a mixture of unknown composition and gave his injections intradermally. It does not appear that von Behring's mixture was very toxic, since in doses of 0.5 c.c. per 100 gms. of body weight, it produced no effect upon guinea-pigs. The advance of the matter to its present position is very largely owing to the work of Park and Zingher, who brought the active immunisation of children and susceptible persons into common use. They utilised a toxin-antitoxin mixture, so adjusted that each 1.0 c.c. of diluted toxin contained 3 L + doses. One unit of antitoxin was then added for each L + dose, the mixture being under-neutralised to an extent that 1.0 c.c. produced paralysis in a majority of 250 gm. guinea-pigs, and 5.0 c.c. produced paralysis and acute death in these animals. On standing, the mixture becomes less toxic and the guinea-pig results less severe. More recently Park has modified this mixture, so that each 1.0 c.c. of the diluted toxin contains only 0.1 L + dose, but this is not so completely neutralised and the amount of free toxin is the same as formerly. It has been found that the immunising efficiency of such mixtures is not in direct ratio to their toxicity.

In certain instances the use of toxin-antitoxin mixtures of this type has been followed by disastrous results. These have been

analysed by O'Brien (1926), who points out that in one case the untoward results were due to disregard of the Danyzs effect. This consists in the fact that when toxin is slowly added to antitoxin, the latter may be saturated by a smaller quantity of toxin than it will neutralise when the two are mixed rapidly and directly. Consequently, where toxin is mixed with antitoxin slowly, or with an interval between the moieties added, the last portion of toxin may remain free and the mixture be dangerously toxic. In the case in question the immunising mixture was prepared without due regard to this effect and an excess of unneutralised toxin resulted.

In a second well-known case, occurring in Concord, U.S.A., in 1924, the toxin-antitoxin mixture was exposed to a very low temperature, and after thawing was found to be dangerously toxic, whilst other bottles of the same batch of material were of normal activity. Glenny, Pope and others (1925) investigated the matter and found that under the influence of cold, and the crystallizing out of water from the mixture, the concentration of phenol, tricresol, or other preservative such as is commonly added, rises until it reaches a concentration at which the antitoxin is destroyed. They found that this effect in the case of phenol could be produced by a concentration of 5 per cent. The matter is an important one to be borne in mind, since in so many institutions the practice of keeping antitoxin and similar products in refrigerators is becoming a routine one. It may be mentioned, in this connection, that a number of writers speak of the dissociation of toxin from antitoxin, in nontoxic mixtures, as occurring on storage, and urge that these should always be used within a short time of their preparation. O'Brien points out that there is no evidence whatever for such an assumption and that, in fact, the effect of continued contact of the two substances under normal conditions is a slight decrease in toxicity, a phenomenon which Park takes into consideration in preparing his immunising mixtures.

Whilst with the added knowledge so gained the likelihood of such unfortunate accidents decreases, it would obviously be desirable if immunisation could be carried out with preparations which under no circumstances could become toxic. In O'Brien's laboratory the immunising mixture as formerly prepared was less

toxic than that of Park, it being required that 5.0 c.c. should fail to kill any of the guinea-pigs injected within a period of 15 days. At the same time an increasing prejudice has grown up against mixtures containing active toxin in any form, and attention has been directed towards the employment of a modified toxin which should be free from even hypothetical risks.

It has been shown by a number of workers, from Ehrlich onwards, that diphtheria toxin can be so modified as to lose its toxicity, whilst still retaining its antigenic powers. The work of von Behring and of Park, which we have just been considering, is a case in point, but, as we have noticed, in cases where toxin is neutralised by antitoxin the possibility of the freeing of toxin under unusual circumstances always exists. On the other hand, the modification of toxin by certain other agents apparently produces a change which is absolutely irreversible. Sal-kowski (1898) discovered that formalin produced such an effect, and Loewenstein (1914) fully investigated the phenomenon and made use of it. The destruction of the toxin by formol is a process whose extent and rate depend upon the concentration of the formol and the temperature at which the reaction takes place. With properly adjusted conditions a very exact degree of attenuation of the toxin can be arrived at. In this country Glenny and Sudmersen (1921) noted that this "toxoid" acted as an efficient immunising agent in the guinea-pig, whilst in France Ramon (1924) made similar observations on the effect of formol and named the toxin thus modified "anatoxin". He also noted its immunising effect upon the guinea-pig and suggested its use in human immunisation, a suggestion which was also put forward by the English workers. Anatoxin was speedily brought into use in France and was also made use of by Park and Zingher in America, and was found to give rise to a satisfactory immunity. The percentage of cases which became Schick-negative after treatment by this method was said by the French workers to be higher than that observed by Park as a result of toxin-antitoxin treatment. The reactions which were recorded seem, however, to have been somewhat more severe than those given by the former preparations. One difficulty in standardising the toxoid, which has been a

stumbling-block in its use, has been met by the introduction of the flocculation method of Ramon (p. 340) which gives a fairly reliable index of the quantity of toxin or toxoid present in any given preparation.

A further advance was made by Glenny and his associates (1924), in the discovery that considerable quantities of antitoxin could be added to toxoid without producing much effect upon its immunising value.

In a typical toxoid-antitoxin mixture of this type there is present in 10 c.c. :

0.1 c.c. of a toxoid, which in doses of 50 c.c. will produce no symptoms in guinea-pigs, its original toxicity having been reduced to about a hundredth of its former value ;

25 to 50 per cent. of the amount of antitoxin necessary to neutralise the toxin in its original unaltered form.

(O'BRIEN.)

In such a mixture, which may be used in human immunisation, there is a double safeguard, in that no actual unaltered toxin enters into its composition, and that even in the extremely unlikely event of toxin becoming freed there is always a sufficiency of antitoxin present to neutralise a large amount of this. All the available evidence, however, points to the change from toxin to toxoid as being an irreversible one.

At the present time the use of toxoid is largely replacing the use of unmodified toxin in all toxin-antitoxin immunising mixtures and, in this country at any rate, a large proportion of the immunisations which are carried out are done by means of Glenny's toxoid-antitoxin, which is the only form of actively immunising mixture issued by one of the leading commercial firms.

A possible future modification of the method may result in the use of the floccules which Ramon (p. 340) showed to occur when toxin and antitoxin are mixed in certain proportions. Sondelli and Serpa (1925), Hartley (1925), and others have found these to be antigenic, and by their use a relatively enormous amount of non-specific matter would be eliminated, to which a certain proportion

of the reactions (following upon the injections) are attributable. Ramon found that the conversion of toxin into anatoxin (toxoid) did not produce any great change in its flocculating ability, and Hartley found that the best antigenic effect was provided by floccules coming from mixtures containing a moderate excess of toxin, the mixture itself being slightly toxic for guinea-pigs. Where floccules were obtained from mixtures containing an excess of antitoxin their immunising effect was low. Glenny and Pope find that the previously quoted results hold, *mutatis mutandis*, for toxoid in place of toxin, so that the possible danger of dissociation, present in floccules made from active toxin, can be overcome by employing toxoid-antitoxin floccules. They further find that the immunity produced by the floccules is established comparatively rapidly and that their antigenic efficiency may be increased by partial destruction of the antitoxin by certain methods such as heating, an observation which had been made by Calmette for the precipitate of neutralised cobra venom in 1908. This work is being actively developed, and should it result in the production of an immunising mixture with a greatly reduced tendency to cause reactions, but which is at the same time efficient and rapid in its action, a very considerable advance will have been made.

A further point, which is suggested by the remarks in the last paragraph, is the desirability of reducing the number of injections required to produce immunity to a single one. It has been found in certain instances that even the minute amount of diphtheria toxin introduced into the skin in the performance of the Schick test may just suffice to swing the balance over from a positive reaction into a negative one. This especially occurs in persons who already have a minimal quantity of antitoxin; the toxin of the Schick test acting in such cases as a secondary stimulus and causing a very considerable production of antitoxin. We thus have explained the anomaly observed in the earlier days of the Schick test, that a child reacting positively might react negatively at a subsequent test, although not immunised in the interval. Individual variations in this respect are wide. O'Brien suggests the future possibility of producing a prophylactic preparation which will be effective upon these lines and give, as a result of a

single injection, an indication of the subject's susceptibility or immunity whilst at the same time containing an efficient dose of the immunising agent, thus very much reducing the number of injections necessary, a great desideratum in the case of children.

### THE RAMON TEST IN STANDARDISING DIPHTHERIA ANTITOXIN

This technique can only be touched upon in its broadest aspects here, since the methods in use nowadays in serum institutes and amongst the commercial firms who manufacture antisera are so highly specialised as to be in their details beyond the scope of the general reader, even in bacteriology.

Although amongst the serum reactions the precipitin test has long been accorded an established place, for years it was current teaching that mixtures of toxin and antitoxin did not give rise to any precipitate, or other *in vitro* immunity reaction. The origin of this mis-statement of fact is unknown to the writer, but he was brought up in its belief. Possibly it may still be in process of being handed from textbook to textbook, as one regrets to observe is that hoary lie that the protoplasm of the lymphocyte, stained by the Romanowsky methods, takes on a " robin's egg blue " !

In 1909 Calmette and Massol made the observation that cobra venom and horse antiserum when mixed gave rise to a precipitate, which appeared at a maximum when the conditions were so adjusted that exact neutralisation was achieved. In an excess of antiserum no precipitate occurred. The precipitate itself was non-toxic. The suggestion was put forward by the authors that this phenomenon might be made the basis of a method of testing the strength of the antitoxin *in vitro*.

All of these findings have since been confirmed for the case of diphtheria and have found some rather remarkable applications. Nicolle, Césari and Debains (1920) extended them to embrace both diphtheria and tetanus, using the rather complicated technique of Ascoli, which consists in the formation of a ring of precipitate when the antitoxin is superimposed upon a column of concentrated precipitated toxin, made up into solid form with gelatin and contained in a test-tube.

Ramon (1922) simplified the matter by observing the precipitate in simple fluid mixtures of toxin and antitoxin. He found that if a series of test-tubes were taken, into each of which was put a constant quantity of toxin, and if to each of them were then added progressively decreasing quantities of antitoxin, and the mixtures shaken and allowed to remain in contact, a precipitate occurred in several of the tubes. Careful observation shows that this commences in a single tube, and that thereafter the zone widens, the tubes on either side after a time showing the same change. The reaction takes place slowly at room temperature, more rapidly in the water bath at 37° to 55° C., but its type and form is the same in both cases. The tube which first shows the appearance of a precipitate is the one in which an exact neutralisation of toxin by the antitoxin has been effected. The tubes on either side of the one first showing this precipitate, which at a later stage also develop precipitates, contain an excess of toxin or antitoxin as the case may be. The reaction is a strictly specific one, occurring only between toxin and the corresponding antitoxin, and is not influenced by the quantity of serum present in the test. Ramon studied various influences which modified this reaction and found, inter alia, that the quantitative relationship between toxin and antitoxin, as indicated by this test, not only held good for fresh toxin, but that when in the process of keeping the M.L.D. of the toxin fell off there occurred no corresponding deterioration in its flocculating power. In other words the process of transformation into toxoid did not diminish its flocculating power, just as Ehrlich had shown it did not diminish its neutralising power *vis-à-vis* antitoxin. Temperatures above 56° C. interfered with both flocculating power and toxicity, and exposure for three-quarters of an hour to 70° C. destroyed both properties. Ramon further found that in the transformation of toxin into anatoxin (toxoid) by formaldehyde the flocculating power of the toxin was not affected.

The possibilities of the Ramon method were speedily grasped by the serum laboratories, especially in view of the economy in animals which could be effected if it were found reliable. Reports have been published by Renaux, in Brussels, Schmidt, in Copenhagen,

Scholz, in Germany, Glenny and Okell, in England; and Bayne-Jones, in America, all of whom agree in the general close coincidence of the values obtained from titration by the Ramon method and from animal experiment. The average error, or difference between the two, as found by these workers in a very large number of experiments, is about 5 per cent., or a little less.

The flocculating power of an antiserum diminishes as the serum is concentrated and the majority of purified antitoxic sera either fail to flocculate or do so in an unsatisfactory manner and after a long delay. Renaux has shown, however, that the addition of a quantity of fresh antitoxic serum restores this quality, and that by titrating such a mixture, with a knowledge of the amount of the fresh serum which has been added and of its antitoxic value, an estimate of the strength of the concentrated antitoxin can be arrived at. Fresh serum which does not contain antitoxin is without effect. Bayne-Jones (1924) has used this method and records that it is satisfactory.

The great use to which Ramon's method is put in the majority of serum laboratories is in the initial stages of antitoxin titration; to give a rough estimate of the quantity of antitoxin in a given serum and as a control upon the animal method of standardisation. It is also of immense use in following the generation of toxin in cultures, and the course of antitoxin production in immunised animals. Once the variation between the *in vivo* and *in vitro* methods has been established for an individual animal this is found to remain remarkably constant, so that very accurate estimates of antitoxin can be made by the latter method. It is nevertheless the practice of many of the best serum laboratories to use the intradermal tests for the later titrations of their antitoxins and the subcutaneous test for the final ones.

It may be noted that the flocculation test is also given between tetanus toxin and antitoxin, but the results here are less constant and less satisfactory than in the case of diphtheria, and the applications of the method are correspondingly restricted.

The occurrence of complement fixation in the presence of toxin and antitoxin is a matter upon which a good deal of difference of opinion has obtained in the past, some observers finding it to

occur, whilst others denied this. Dean, who in previous work has shown that in precipitation and complement-fixation tests the maximum reaction depends upon the mixture of antigen and antibody in optimum proportions, has reconsidered the question for diphtheria toxin and antitoxin in the light of Ramon's work. He finds (1927) that in this reaction the fixation of complement is a regular phenomenon, but here also is dependent upon optimum proportions. It occurs only within a narrow zone in mixtures of different dilutions of toxin and antitoxin, which, however, bears a definite relationship to that in which flocculation occurs.

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## CHAPTER XVII

### RECENT WORK UPON THE ANAEROBIC ORGANISMS

THE state of our knowledge of the anaerobic bacilli has been completely revolutionised by the studies made during the late war. Prior to that time knowledge was limited and imperfect, and confusion abounded. The urgent necessities of the war period resulted in a complete overhaul of the whole subject. technique was improved, knowledge widened, confusion dissipated, and information enormously extended. These advances were in the main due to the labours of the British systematic bacteriologists, such as Henry and McIntosh; to the discovery of new pathogenic forms by the French workers, Weinberg and Séguin, and to the serological studies of Bull, and the technical advances introduced by McIntosh and Fildes and by Brown.

As is well known to-day, and as was emphasised early in the modern phase of anaerobic work by Miss Robertson (1916), the difficulties in obtaining anaerobes in pure culture are considerable and are very unlikely to have been overcome by a majority of the older workers, whose technical equipment was not of the highest order. From these difficulties sprang errors and contradictory findings which left a host of conflicting statements for the confusion of their later followers, in fact it was not until much of the old work was disregarded, and a fresh start made, that these difficulties were resolved. They sprang chiefly from two sources. incomplete descriptions, and manifestly contaminated cultures. The latter difficulty is the one which cloaked the work of Klein upon his *B. enteriditis sporogenes*, which up till recently was a name found in every bacteriological text-book. Von Hibler found that Klein's cultures contained at least two anaerobes, and it is probable that he made an underestimate. In the light of later

knowledge it is certain that these were mixtures, as Von Hibler suspected, and contained, amongst others, both *B welchii* and *B sporogenes*. A similar difficulty, but due more to the lack of a description sufficient for certain identification, surrounded Pasteur's *Vibrion septique*. This organism, which Pasteur isolated from animals dying from what was supposed to be anthrax, a contention which he disproved, was highly pathogenic for the guinea pig, producing serious sloughing and gaseous phlegmon. Koch, working at the same time at the same problem, described a similar organism under the name of *B œdematis maligna*, which he stated was the same as Pasteur's organism, and the statement was very generally accepted without question. Koch's organism, however, differed in pathogenicity from Pasteur's and, though it will never be identified with certainty, was most probably *B. sporogenes*, or else his cultures were contaminated with this. Pasteur's organism, which thus became merged into the identity of *B. sporogenes*, has latterly been rehabilitated and once more restored to an individual place under Pasteur's original name, *Vibrion septique*, an organism of the saccharolytic group and very highly pathogenic.

. . . . .

The more important systematic portions of this new work have already found their way into the standard text-books and can be read in their original form in the papers of Henry and McIntosh, in the Reports of the Medical Research Committee, and in the monograph of Weinberg and Séguin. Only certain points in recent work will therefore be considered here.

### B. WELCHII

This, which is the most widely diffused and one of the most active members of the saccharolytic group of anaerobes, is of especial interest on account of the frequency with which it participates in the anaerobic infections, and also on account of its well-developed toxic properties, which have been only recognised of recent years and whose effects are possibly not yet fully under-

stood. Both Simonds and Henry recognise four types, on the grounds of fermentation of glycerin and inulin :—

				Inulin	Glycerin
Type I.	.	.	.	—	—
„ II.	.	.	.	—	+
„ III.	.	.	.	+	—
„ IV.	.	.	.	+	+

but these differences do not appear to be of importance for the behaviour of the organisms in infective conditions. The bacillus is essentially a saccharolytic type, not attacking proteins if carbohydrates are available, and establishing itself best in the body in sites in which there is an abundance of such materials, *e.g.*, muscle and liver. It is on account of this feature that wounds involving a considerable area of muscle are those most prone to be followed by gas gangrene. The organisms, when washed free of culture media and suspended in saline, are as a rule non-pathogenic, but broth cultures when injected into the thigh muscles of guinea pigs produce a spreading phlegmon and bubbles of gas in the tissues. McNee and Dunn showed that in man the infection tends to spread rapidly along the sheaths of the muscle fibres, stripping these from the sarcolemma and distending them with gas, whilst the devitalised muscle undergoes rapid degenerative changes. In this way the infection in a very short space of time will involve the whole length of a muscle, from the site of infection to that of the muscle's origin.

Rosenthal, in 1916, recognised that a specific toxin was produced by this organism and was able to make a horse antiserum, which, however, was only feebly protective. Klose, the same year, prepared a weak toxin from *B. welchii* and made an antitoxin which would protect against three lethal doses of *B. welchii* culture: his toxin was not very potent. Weinberg (1916) also prepared an antiserum by the injection of washed living bacilli, which proved to have a powerfully protective effect, this was no

doubt due in great part to its antitoxic properties. It remained for Bull and Pritchett (1917) to really demonstrate the powerful toxogenic action of the organism and clear the way for the production of an efficient antitoxin. Bull prepared his toxin, in the first instance, by growing the organism in glucose broth to which a considerable mass of fresh rabbit muscle had been added. He utilised a strain or organism whose virulence had been exalted by repeated passage on the pigeon—an animal particularly susceptible to *B. welchii*. By such means he produced a toxin capable of killing the pigeon in doses of 0.1 to 0.01 c.c. De Kruif, Adams and Ireland (1917) confirmed this work, and in a similar medium produced a toxin 0.1 c.c. of which killed a 300-gramme guinea-pig upon intramuscular injection. All the workers at this subject have found a great deal of variation between the toxin-producing power of different strains of the bacillus. The method of Bull is not one which lends itself well to commercial practice, since the aseptic manipulation of large masses of fresh rabbit muscle presents great difficulties, and subsequent workers have found that an efficient toxin can be prepared by the substitution of cooked meat. The English workers (M.R.C. Report, 1919) found that a peptone broth, containing minced horse muscle and 0.2 per cent. of glucose, gave good results. The favouring effect of glucose had been reported by Bull, who had also shown that in the presence of an excess of carbohydrate the high acidity developed in the medium had a deleterious effect upon the toxin. Weinberg (1927) employs a peptic digest of liver, to which minced beef is added and 1 per cent. of glucose.

The Welch toxin is a complex substance which contains, *inter alia*, a powerful hæmolysin and a substance causing muscular necrosis, which Henry proposes to call "myotoxin." The effects of this may be well shown by the intramuscular injection of toxin into laboratory animals. Weinberg and Nasta have found that these two elements are produced in different ratios by different cultures, some being highly hæmolytic and others producing chiefly the non-hæmolytic moiety of the toxin. They have also found that it is possible to dissociate the two components by saturating the hæmolysin with red cells. Under such circum-

stances the total toxicity of a given sample becomes much reduced. There is also evidence that the toxin contains a substance which powerfully stimulates involuntary muscle. The effect of the toxin in acute gas gangrene would appear to be chiefly an aggressive one since, as we have already said, washed Welch bacilli are relatively non-pathogenic, but, in the presence of toxin, they become powerfully activated and produce fatal infections. In the presence of a non-lethal dose of toxin a very few bacilli are sufficient to set up a fatal gas infection.

**B. Welchii Antitoxin.**—The production of this for therapeutic purposes was first carried out by Weinberg in France, and Bull and Pritchett in America. Bull titrated his serum upon the pigeon; Weinberg upon guinea-pigs, using as a unit the amount of serum necessary to protect a standard guinea pig from a minimum lethal dose of toxin. In this country mice have been largely used, as in them the minimum lethal dose is readily ascertained and the use of such animals is a considerable economy. During the war sera of this nature were prepared by a number of the combatants, and in most cases were made polyvalent, including as a rule *Vibrio septique* antitoxin and, in the case of Weinberg's serum, *Bacillus œdematiens* antitoxin. Recently Weinberg and Prévot have attempted to use formalinised toxin, following the practice successfully employed in diphtheria. They found that the addition of formol destroys the hæmolytic activity of the toxin and also its general toxic effect. In their hands such a modified toxin has proved to be quite suitable for the preparation of anti-toxic *B. welchii* serum. Dalling and Mason (1926) also found that formalinised toxin, stored toxin whose activity had become enormously reduced, and toxin-antitoxin mixtures, all acted satisfactorily in producing active antitoxic immunity in horses.

In a general way it may be said that the remarks made upon the toxigenic effects of *B. welchii* may also, *mutatis mutandis*, be applied to the other important pathogenic gas-producing anaerobes *B. œdematiens* and *Vibrio septique*. In the case of the last-named organism, Miss Robertson showed the existence of at least four serological types. From the point of view of toxin production, however, these all behave the same. Although a less frequently

encountered anaerobe in human wound infection than *B. welchii*, the *Vibrio septique* is a highly pathogenic organism, the results which it produces in the experimental animal being even more severe and fatal than those caused by *B. welchii*. The organism is very closely allied to *B. chauvæi*, but is distinguished from it by the fact that *B. chauvæi* ferments saccharose but not salicin, whereas the opposite is the case with *V. septique*. The specific toxins of the two organisms are also quite distinct. Dale has especially studied the effects of the toxin of *V. septique*, which is capable of producing the death of animals with extreme rapidity, and finds that it has a special action on the heart somewhat like that produced by poisonous doses of digitalis, viz., an initial rise in blood pressure followed by cardiac irregularity and a fall of the vascular tension. The effect is a direct one, and is not influenced by section of the vagus.

The experience of the war was that in cases of traumatic gas gangrene, which were practically always mixed infections, the use of polyvalent sera was imperative. In the time which has elapsed since, the demand for such sera has waned, but certain recent developments in civil practice have again turned attention to their possible value in other directions.

### INTESTINAL OBSTRUCTION AND PERITONITIS

In acute peritonitis the secondary development of paralytic ileus brings the disease into close clinical similarity with acute intestinal obstruction, both of which result in a profound toxæmia. The toxæmia arising from such conditions, and the toxic products developing in closed intestinal loops, have been the subject of a large amount of research, especially in America. It is commonly conceded that in the actual disease an absorption of toxic material occurs from the bowel proximal to the point of obstruction, and is aided by the concomitant dehydration to which the patient is exposed. The importance of preventing this absorption, by drainage, is generally recognised, as is also the danger from toxic absorption which arises from the sudden passage of a mass of obstructed bowel contents into the

healthy area distal to the obstruction, which may occur when this is relieved. It is also very widely agreed that these toxic substances are the result of bacterial decomposition of the intestinal contents, although Whipple and his co-workers are notable dissentients from this point of view. B. W. Williams (1926) has been struck by the resemblance of the clinical picture in this condition to that seen in the toxæmic cases of gas gangrene, and suggests that here, too, the intoxication may be due in large measure to the absorption of *B. welchii* toxin, which he thinks is in most cases developed in the lower ileum. In the vomit in cases of acute obstruction he found *B. welchii* eleven times out of twelve specimens, and in advanced cases of peritonitis nineteen times out of twenty cases. In examining the obstructed intestinal contents, for the presence of free Welch toxin, Williams injected mice with the Berkefeld-filtered material obtained both from human cases and experimentally obstructed dogs. Fifty-four mice in all were injected with material from seven cases, and of these twenty-eight died. Twenty-seven control mice were simultaneously inoculated with a small quantity of *B. welchii* antiserum, and all survived. In support of his view of the genesis of this acute toxæmia, Williams also points to the marked evidence of hæmolysis in the tissues of patients who have succumbed to these conditions under circumstances in which the influence of streptococci hæmolysins can be excluded. Whilst it might be expected that such a toxin should be formed under the conditions obtaining in obstruction of the gut its ready absorption will not be conceded *à priori*, very diverse views being held upon the ease and extent to which toxic absorption from the intestines may occur.

The view here developed was put to a clinical trial by the use of an anti-gas-gangrene serum, containing antitoxins against both *B. welchii* and *V. septique*, in a series of 256 cases of acute appendicitis, the antiserum being used only in such cases, eighteen in all, as appeared to be "going wrong." The nett mortality for this series was three, *i.e.*, 1.17 per cent. During the same period the mortality in another series of 111 cases in the same institution, in which serum was not used, was 6.3 per cent. A further trial was made in cases of acute intestinal obstruction. Fifty-four cases

were observed in the same way, and treated with *B. welchii* anti-toxin when this seemed to be indicated. The nett mortality was five, *i.e.*, 9·3 per cent. In as nearly as possible a parallel series of 214 cases, occurring in the same hospital between 1919–23, the mortality was 24·8 per cent.

Williams remarks that in most of the cases in which serum was given the clinical improvement was notable, and the distension of the gut rapidly subsided. He is very guarded in his appraisal of its effects, but thinks that it is a valuable adjuvant to the ordinary surgical measures in controlling toxæmia and relieving distension. The doses of serum were large, an initial one of 80 c.c. being given intramuscularly, with, in extreme cases, a further 40 c.c. intravenously. Thereafter 40–80 c.c. were given daily until the bowels became spontaneous and regular in action and all distension gone or nearly so. It is interesting to recall that in the very considerable amount of work that has been done in the past upon the toxicity of materials developing in closed intestinal loops, the possibility that *B. welchii* might be a contributor to this effect appears to have been very largely overlooked. The views put forward by Williams have attracted considerable attention, and at the present time the use of anti-*B. welchii* serum is receiving an extended trial in the conditions in which it has been advocated.

### THE HÆMOLYTIC EFFECTS OF *B. WELCHII*

One of the products of the growth of *B. welchii* is a powerful hæmolysin. Henry, who has studied this toxin with some care, finds that it is capable of preservation in the dried state, and also in culture filtrates kept in the ice chest. It is destroyed by heating for thirty minutes to 60° C. The toxin has a solvent action upon the red cells of the mouse, pigeon and fowl, and, to a less extent, upon those of man, the guinea-pig, rabbit and sheep. The corpuscles of the horse are resistant. Unlike most bacterial hæmolysins, that of *B. welchii* is neutralised by Welch antitoxic serum.

The view has been growing up of recent years that the chronic hæmolytic toxæmia, which most workers agree to be the basis of

pernicious anæmia, may have its origin in an abnormal overgrowth of *B. welchii* in the small intestine. Whilst, from William Hunter onwards, the possibility of a portal hæmolysis has been freely admitted, the exact cause of this has by no means been agreed upon. The well-known achylia of pernicious anæmia is associated with the presence of a large variety of organisms in the stomach itself and therefore, presumably, with a constant and excessive bacterial content throughout the whole of the small intestine; a fact which has been actually demonstrated by Van der Reis (1922) and Seyderhelm (1924) by ileostomy. The organism to which most attention has been directed in the past is the hæmolytic streptococcus, but recently Welch's bacillus has come in for greater consideration, as a result of better knowledge of its active hæmolytic properties and of recent work upon the anæmia produced therefrom. Moench, Kahn and Torrey, examining the stools in pernicious anæmia, found an excess of viable organisms of which *B. coli*, *streptococci* and *B. welchii* were the chief members, with, at times, *B. acidophilus*. They were impressed by the predominance of the actively saccharolytic group, and especially by the dominance of *B. welchii*. These results have been confirmed by Davidson (1927), who found that, as compared with the normal, the quantity of streptococci in the stools in pernicious anæmia was  $\times 100$  to  $\times 1,000$ ; of *B. coli*  $\times 1,000$  to  $\times 1,000$ ; and of *B. welchii*  $\times 100,000$  to  $\times 1,000,000$ .

Kahn and Torrey (1925) injected three monkeys with repeated doses of a *B. welchii* hæmotoxin, and produced an acute anæmia with high colour index and the presence of poikilocytes, anisocytes, polychromatophilic and punctate basophilic red cells, and nucleated red cells including megaloblasts; and Patterson and Kast (1925) produced similar results in rabbits with cultures of *B. welchii* injected intramuscularly. The last-named workers also experimented with *B. bifermentans* and *B. sporogenes*, but did not obtain anything like such marked effects. Cornell (1925), by the intra-splenic inoculation of cultures of *B. welchii*, produced a chronic infection in rabbits with well-marked blood changes resembling those of pernicious anæmia.

The fact that a known hæmotoxin, such as that of *B. welchii*,

will produce a severe anæmia, with changes simulating those of human pernicious anæmia, is not by itself of any great significance, since it is well known that any of the common agents of blood destruction will do the same, provided they are of sufficient activity and the experiment is so arranged to allow the necessary time factor for the production of regenerative changes to come into play. The experiments of Cornell are more suggestive, since the condition here was produced by bacteria existing in the tissues, and not by the injection of a bacterial product. Some further experiments of Kahn and Torrey (1927), who found that the injection of *B. welchii* toxin resulted eventually in the production of an antitoxic immunity, would also be very much to the point if they were confirmed and extended. These workers found that when given by the mouth, the toxin was either destroyed or not absorbed, but that if the mucosa were damaged, by giving sodium fluoride, the weekly ingestion of the toxin was followed by a severe anæmia which, they state, was not associated with any antitoxic immunity. It may be said that their experiments were only two in number, and involved a very drastic procedure in the monkeys, which alone produced a not inconsiderable anæmia in the controls. They cannot, therefore, be regarded as more than suggestive. A failure to produce antitoxic immunity is an essential condition in any such theory of pernicious anæmia; but it would be a very remarkable finding, in the case of a hæmolytic toxin capable of engendering this, in view of such experiments as those of Ramon and Grasset (1926), who showed that tetanus toxin could be absorbed from the alimentary canal and thereby provoke the appearance of antitoxin, and also those of Weinberg and Goy (1928), who obtained the same results with *B. botulinus* anatoxin. In fairness to Kahn and Torrey, it must be noted, however, that Ramon and Zoeller (1926) could not confirm their guinea-pig experiments, using relatively small doses of tetanus anatoxin, in a few experiments made upon man. In the *B. welchii* theory of pernicious anæmia it will have to be conceded that the absorption of toxin must be a slow and chronic process, and the amount in play at any one time, therefore, presumably small.

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## LAMB DYSENTERY

There occurs in newly-born lambs, in various parts of the country but especially upon farms in the Scottish border counties, from whence it appears to have spread both north and south, a severe and fatal diarrhoea which produces a heavy annual loss of lambs in the affected districts. The mortality varies from year to year, but on an average often causes a loss of 10 to 20 per cent. of all lambs born. The case mortality is very high, death being the rule. The lesion in animals dying of the disease is an acute inflammatory enteritis, with hæmorrhages into the mucosa and localised areas of necrotic inflammation; the condition in acute cases may involve the whole length of the bowel.

This disease has been investigated very extensively in this country by Dalling, and various co-workers, since 1921. Dalling, and Gaiger and Dalling, originally believed the infection to be traceable to a type of *B. coli*. They prepared an antiserum to this, which was administered to newly-born lambs, but the results were not satisfactory. In 1928 these authors began to revise their conclusions, and to realise that an anaerobic organism was of constant occurrence in the intestinal lesions, gradually their view changed, so as to lay greater and greater stress upon this organism, and with the adoption of such a view their experimental results progressively improved. By feeding young lambs with a mixture of the coliform organism and the anaerobe, which had been identified with *B. welchii*, they were able to produce the disease experimentally, and somewhat later Dalling (1926) found that feeding with a culture of *B. welchii* alone produced the typical disease in a fatal form. Injection of the *coli* antiserum did not prevent such a result, but injection of *B. welchii* antiserum protected the animals. The disease could also be produced experimentally by the intravenous injection of the organisms, and successfully prevented by the use of Welch antitoxin.

As a result of these experiments, Dalling abandoned his previous view that the coliform organism was of any significance in the disease and now looks upon the anaerobe as its ætiological agent. The exact relationship of this organism to *B. welchii* is unsettled. It is not absolutely identical with it, since pure *B. welchii* anti-toxin will not completely neutralise the toxins of Dalling's

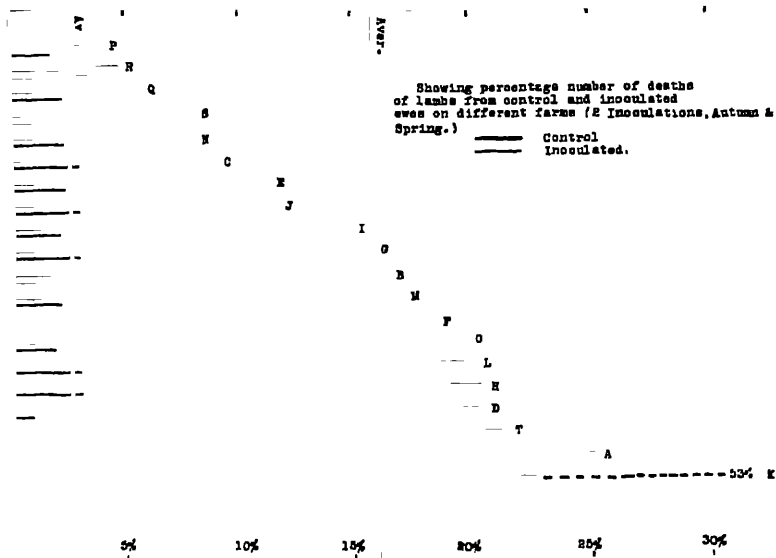


FIG 22.—The results obtained in the preventive treatment of lamb dysentery by inoculation of the ewes. (Dalling, 1926)

organism ; although in the converse experiment the Welchii toxin is completely neutralised. There is, therefore, an additional element in the toxin of the lamb dysentery anaerobe, which is absent from the pure *B. welchii* toxin. O'Brien suggests that Dalling's organism is a mixture of *B. welchii* with an undescribed anaerobe, but so far the separation of the additional factor has not been accomplished.

Attempts at prophylaxis have been made in two directions. On the one hand pregnant ewes have been immunised with *B.*

*welchii* toxin-antitoxin mixtures shortly before parturition, in the expectation that a sufficiency of antibodies would be transmitted to the newly-born lambs to tide them over the period of danger. In the earlier work, this was associated with the use of a *B. coli* vaccine, but later this was omitted without the results being adversely affected. A number of carefully controlled experiments have been carried out, and the results would appear to be strikingly satisfactory (Fig. 22), the mean morbidity from the disease being reduced from about 16 per cent. of lambs born in the uninoculated group to about 3 per cent. in the case of lambs born from inoculated ewes. Dalling and his collaborators have also studied the effect of different methods of inoculation, and find that double inoculations, either in autumn and spring, or twice in the spring, give better results than a single injection at either of these times. The maternal immunity is not, it would appear, of very long duration, falling off rapidly within a few weeks of the prophylactic injection. Since the concentration of antibodies in the lambs at birth is the same as that in the maternal fluids, it is important that the immunisation should be timed so as to give as high a degree of protection to the lambs as possible. The immunity in the lamb, according to Dalling, Mason and Gordon (1927), lasts for about a week.

On the other hand, a series of prophylactic observations have been made upon lambs passively immunised by means of *B. welchii* antiserum, injected into them as soon after birth as possible. In a series of cases in which this method of prophylaxis was followed the mortality amongst 1,122 inoculated lambs was 0.44 per cent., whilst that amongst 1,241 uninoculated lambs, upon the same farms and during the same period, was 17.16 per cent.

It should be mentioned that as a result of an independent investigation of the disease in Lancashire, Hare and Glynn (1927) take the view that upon the available evidence there is no proof that the condition is a contagious disease or an infection at all, and they are unable to incriminate *B. welchii* or other organism in its causation. They point out the close and constant connection between the age of the lamb and the period at which the attack supervenes, and suggest an analogy with

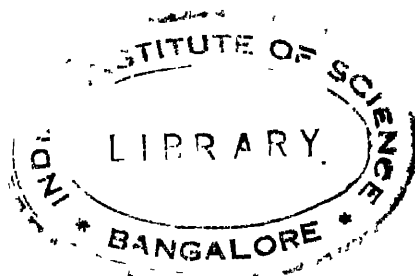
*melæna neonatorum*, in which disease serum of any kind is said to have a curative effect irrespective of any antibody content. They therefore suggest that the favourable results obtained by Dalling and his collaborators in the treatment of the condition may be non-specific ones.

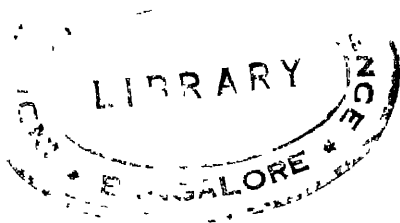
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